

8974-21

THE AMERICAN NATURALIST

Established 1867

A BI-MONTHLY JOURNAL

Devoted to the Advancement and Correlation
of the Biological Sciences

L. C. Dunn, *Managing Editor*. Columbia University.

EDITORIAL BOARD

(in process of formation; partial listing)

G. Evelyn Hutchinson, Yale University.

Thomas Park, University of Chicago.

G. Ledyard Stebbins, Jr., University of California.

Conway Zirkle, University of Pennsylvania.

Jaques Cattell, *Publisher*.

Published for
THE AMERICAN SOCIETY OF NATURALISTS
by
THE SCIENCE PRESS
Lancaster, Pennsylvania
1951

THE SCIENCE PRESS
LANCASTER, PA.

STAT. H.B.
V. 98

THE AMERICAN NATURALIST



THE AMERICAN NATURALIST

Vol. LXXXV

January-February, 1951

No. 820

EDITORIAL

With this, its 85th volume, THE AMERICAN NATURALIST becomes the journal of the American Society of Naturalists. Thus at a ripe age in the life of each party there is consummated a union between a periodical and a society which had long shared both name and interests yet had no formal connection.

THE AMERICAN NATURALIST* which began publication in 1867 had been edited and published for many years (1907-1939) by Dr. J. McKeen Cattell, in association with his son, Mr. Jaques Cattell, and since 1943 (except for a brief interlude) by Mr. Jaques Cattell. It was he who in 1941 sought the collaboration of the American Society of Naturalists which appointed a rotating board of consulting editors, while Mr. Cattell remained as editor and publisher. During 1950 the Society, at Mr. Cattell's request, designated an editor, and after polling its members decided by a very large majority to adopt THE AMERICAN NATURALIST as its journal. The agreement which was ratified unanimously by a membership meeting held in Columbus Ohio on September 12, 1950 gives the Society complete editorial control and provides a subscription to THE AMERICAN NATURALIST for each member of the Society in good standing. In effect, questions of content will be decided by the Society through its editor and editorial board; questions affecting the form and frequency of publication and all business matters will be decided by the publisher; but major questions of any kind will be decided only after consultation between the editors and the publisher.

The American Society of Naturalists was organized in 1883. Its constitution states that its object shall be "the association of working naturalists for the discussion, advancement and diffusion of knowledge concerning the broader biological problems, including organic evolution, thus serving to correlate the various biological sciences into a common philosophy of biology." Its function "as a general rather than a specific society" was confirmed in 1931 by the adoption of a report on policy which has governed the Society's activities until this year. Now to its usual functions of ar-

*The history of THE AMERICAN NATURALIST has been described by Professor E. G. Conklin in the issue of January, 1944 (Volume 74, pp. 29-37). This issue contains also two other papers prepared for the 75th anniversary of the journal by L. C. Dunn (The Naturalist in America) and Paul B. Sears (The Future of the Naturalist).

ranging annually a symposium, and an address by its president is added the editing of a journal.

The future character of *THE AMERICAN NATURALIST* will be determined by three factors: the objectives of the Society, the past history or traditions of *THE AMERICAN NATURALIST*, and the changing needs of biology and biologists. The first two have been in general conformity for most of the life of the Society, that is to say, the journal actually did what the Society would have had it do. The reason for this parallelism is obvious: both were responses to the great surge of interest in natural history and in general biology for which the motive force and the unifying idea were provided by the theory of organic evolution. During the first half of the existence of *THE AMERICAN NATURALIST* and the early years of the Society, the center of interest was in taxonomy, description, exploration, field and museum work, and in methods in support of these; whereas in later years, as genetics became increasingly useful in the experimental study of the factors of evolution, this field came to occupy an increasing amount of space in the journal and of interest in the Society. Likewise a prominent characteristic of the journal came to be its publication of general addresses and of papers presented at symposia of various biological societies including those sponsored by the Society as its chief activity. Thirdly, *THE AMERICAN NATURALIST* for many years provided a medium for the publication of short reports and discussion of new biological research of the same kinds as those which interested the members of the Society.

Current correspondence with biologists indicates that these are all considered to be useful functions of the journal, of which none except the publication of research reports in evolution and in genetics is adequately served by other journals. In addition it appears that one function, only partly served by *THE AMERICAN NATURALIST* in the past, is felt as a distinct need by many biologists. The one word which serves as the common denominator for the various forms in which this need has been expressed is synthesis. The kind of synthesis which biologists want is that which arises out of a really adequate analysis both theoretical and factual, and this is most likely to occur in connection with a specific problem to which someone has devoted, with the enthusiasm which accompanies discovery, both the thought and the labor of actual investigation. Biologists probably intend the same meaning when they say they want to see the problem in its larger context, in the perspective of some general biological view.

We may say that *THE AMERICAN NATURALIST* should evolve in these three directions: (1) To provide a medium for the publication of general addresses and essays and the symposia of biological societies which in the opinion of the editorial board contribute substantially to the main purpose of the Society in "correlating the various biological sciences"; (2) To publish quickly brief reports of new research of general biological interest and brief comments and discussion of these, including comments and criticism of material published elsewhere; (3) To publish papers in which the chief interest inheres in the theoretical interpretation and the synthesis referred to above.

To say this however is to express a hope rather than to create this kind of journal by fiat, for what is to be published must obviously be a reflection of what biologists will provide. Certainly until the membership of the editorial board is completed and has some experience, no radical or permanent changes need be envisaged.

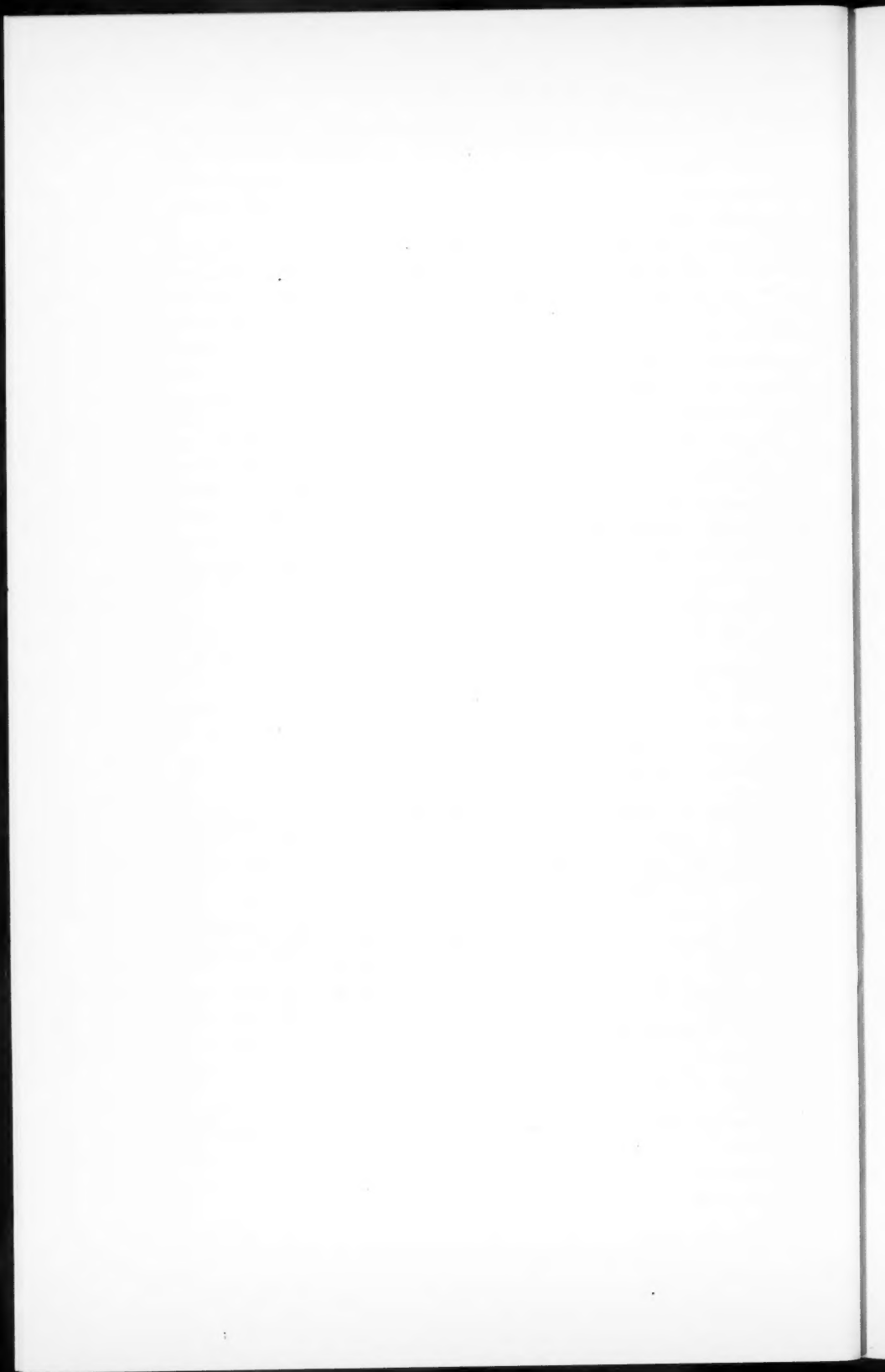
In the meantime the nucleus of the editorial board will be guided by the views of members which have been expressed in meetings and correspondence. THE AMERICAN NATURALIST may be expected to move away from the publication of extensive data papers for which adequate outlets already exist; to become more hospitable to criticism, comment and brief reports especially when these can be put in the form of letters to the editor, and to increase its invitations to naturalists to review briefly new advances in their fields. The editors will be glad to receive manuscripts of these kinds and, for the present, of the kinds which the NATURALIST has published in the past, on any field of biology or natural history. In these ways, it hopes to serve not only members of the American Society of Naturalists but biologists in all countries and has no intention of favoring either members or Americans in choosing material for publication.

THE EDITORS

To Members of the American Society of Naturalists:

This issue is being sent to all members of record whether or not they have paid membership dues for 1951. Subsequent issues can be sent only to paid-up members. Members who have not yet done so will please remit dues (\$5.00 including subscription) to:

PROF. D. F. POULSON, *Treasurer*
Osborn Zoological Laboratory
Yale University
New Haven, Conn.



INTERBREEDING OF NATURAL POPULATIONS OF VERTEBRATES¹

W. FRANK BLAIR

The University of Texas

INTRODUCTION

The rate of divergence of natural populations of both plants and animals is dependent on the amount of gene flow between the populations. Reduced gene flow obviously favors differentiation, while free gene exchange tends to prevent divergence of populations. The interbreeding, or hybridization, of different populations in nature permits gene exchange between such populations, and the amount of gene transfer is dependent on the amount of hybridization. Introgressive hybridization of subspecies, species, and genera has been given much weight as a major cause of the variability in existing populations of plants by Anderson and Hubricht (1938), Anderson (1948, 1949), Heiser (1949) and others of this same group. For purposes of comparison, it seems profitable to discuss the occurrence of, the apparent basis for, and the evolutionary implications of hybridization in nature between populations of mammals and between populations in other vertebrate groups. No attempt has been made, however, to review all of the widely scattered literature on hybridization of vertebrates. Most of my examples will be drawn from a few groups of vertebrates, including *Peromyscus* among the mammals, the acaudate amphibians, and certain families of fishes.

The stage of speciation of two populations will presumably affect their potential rate of gene exchange. The probable course of speciation will be reviewed, therefore, as a background for discussing the interbreeding of natural populations. We believe that geographic isolation of populations is usually the initial step in speciation (see also Mayr, 1942, 1947). Once geographic isolation occurs, the populations are free to diverge in many ways. They may come to differ morphologically, physiologically, and psychologically, and their hereditary materials may eventually become so different that the two populations would no longer produce hybrids. They may come to differ in habitat preference, in place of breeding, and in their response to various environmental factors associated with the initiation of reproduction. If any combination of these characters will act to prevent

¹Read at a symposium on "The Role of Introgression in Evolution," before The Society for the Study of Evolution, Columbus, Ohio, September 11, 1950.

interbreeding, that is, as isolating mechanisms, between the two populations, these populations can come to occupy the same range without losing their distinctness through interbreeding. Most modern biologists would define such reproductively isolated populations as species. Because evolution is a continuing process, we can expect at any moment in time to find natural populations in all stages of speciation.

If the species is defined on the basis of reproductive isolation, there should be a fairly sharp cleavage between cases of the interbreeding of species and the interbreeding of populations that have not reached this stage of evolution. In the case of species, hybridization involves the breakdown of the mechanisms of reproductive isolation. The fact that isolating mechanisms do break down under some circumstances has necessitated a revision of the biological definition of species to admit the possibility of some gene exchange (see Mainland, 1942; Mayr, 1949). In the case of intraspecific populations, interbreeding occurs between populations that have not developed effective isolating mechanisms. Our position on the classification of natural hybrids is that sympatric species have demonstrated their stage of speciation by their co-existence in nature (see also Mayr, 1942). Hybrids between such populations would be regarded unquestionably as interspecific hybrids, resulting from the breakdown of the isolating mechanisms that act to maintain the distinctness of the two populations. Classification of geographically separate populations is difficult, because the stage of speciation is unknown in the case of most of these populations. The conventional taxonomic practice is to classify such isolated populations as species if there is morphological differentiation. This practice accounts for much of the looseness of usage of the term interspecific hybrid in the current literature.

A classical example of the weakness of morphological criteria in evaluating isolated populations is given by Clausen, Keck, and Hiesey (1947). A new genus on morphological grounds proved, on breeding experiment, to be a subspecific population of a previously described species, *Layia glandulosa*. Mayr's (1948) suggestion that all isolated, allopatric populations be called subspecies until proved otherwise has some merit, but Mayr agrees that, "it is just as serious an error to call a population a species if it is really only a subspecies than to call it a subspecies if it is a species." Whatever expedient we use for purposes of classification of such isolated populations, we should keep in mind that such populations may or may not have acquired intrinsic isolating mechanisms. Previously isolated populations that have extended their range to interbreed in a zone of contact are easier to classify. The interbreeding of these populations in the zone of contact is evidence that these populations were in an incipient stage of speciation. We would regard hybrids between such populations as intraspecific, not interspecific hybrids.

In the following discussion, we will treat separately cases of interspecific hybridization and cases of interbreeding between the various categories of intraspecific populations.

HYBRIDIZATION OF SYMPATRIC SPECIES

In discussing hybridization of sympatric species, we are interested in whether or not such species can hybridize, in whether or not they do hybridize in nature, in the frequency with which hybrids are produced, and in the evolutionary significance of such hybridization.

Fertility Between Species

The potentialities for natural hybridization of species have been shown to exist in all vertebrate groups that have been adequately studied. It has become increasingly evident in recent years that many closely related species are at least partially interfertile. Members of the same species group in *Peromyscus* can be crossed in the laboratory to produce at least some fertile F₁ hybrids when no choice of mates is given (see Dice, 1933, 1937; Dice and Liebe, 1937; Watson, 1942; W. F. Blair, 1943a). There is similar evidence from widely scattered groups of vertebrates, including: toads and tree-frogs (A. P. Blair, 1941b); ranid frogs (Moore, 1946); cyprinodont fishes (R. R. Miller, 1948). Numerous cases of interspecific hybrids in birds are summarized by Mayr (1942). More interspecific hybrids have been produced in the laboratory in *Drosophila* than in any other group of animals. Patterson (1942) listed thirty-one interspecific hybrid combinations, and he informs me that the number now has more than doubled. It seems to be generally true that diverging populations of animals usually acquire mechanisms of reproductive isolation before they have diverged genetically to the point of intersterility.

So-called intergeneric hybrids are not distinguishable from interspecific hybrids. We are dealing in both cases with hybrids between representatives of different populations of animals. Genera are subjective units of classification. Most intergeneric hybrids are described from fishes and birds, the groups of vertebrates in which there has been the greatest amount of taxonomic splitting.

Occurrence of Sympatric Hybrids

Because closely related species are frequently interfertile, the probability of species populations hybridizing in nature depends largely on the effectiveness of the isolating mechanisms that prevent fertilization of the eggs of one species by the sperm from another, related species. Because the mode and pattern of reproduction vary greatly within the vertebrates, it is not surprising that different complexes of isolating mechanisms have been developed in different groups. It is to be expected that some groups have developed more effective complexes than have others. Present information about complexes of isolating mechanisms in several groups of vertebrates has been summarized in table 1. Some significant conclusions can be drawn from this comparison. The most important is probably that in every case studied there are several mechanisms involved in keeping the species separate. The combination of mechanisms varies from group to group, but complexity of factors is characteristic of all groups. The complex of factors appears more susceptible to breakdown in some groups than in others.

TABLE 1
COMPLEXES OF ISOLATING MECHANISMS REPORTED FROM SEVERAL GROUPS OF VERTEBRATES

Group	Mode of reproduction	Isolating mechanisms						Authority
		Habitat preference	Breeding season	Call	Mating preference	Courtship pattern	Sexual dimorphism	
Bufo (amphibia)	Ext. fertilization; breeding swarms.	Partial	Partial	Yes	No	No	Slight	A. P. Blair (1941a)
Hylidae (amphibia)	"	"	"	"	"	"	"	A. P. Blair (1941b)
Peromyscus (mammals)	Int. fertilization; permanent pairs.	"	No	No	Yes	Unknown	No	Papers by Dice, W. F. Blair, Blair and Howard
Centrarchidae (fishes)	Ext. fertilization; nest in colonies.	"	Little or none	"	Probably	Yes	Some	Hubbs and Hubbs (1932), Bailey and Lagter (1938)
Cichlidae (fishes)	Ext. fertilization; pair formation and care of progeny.	"	?	"	Postulated	?	?	Kosswig (1947) Trewavas (1947)
Poeciliidae (fishes)	Int. fertilization.	Slight	?	"	Yes	Yes	Yes	Haskins and Haskins (1949)

Groups that practice external fertilization and form breeding swarms are more apt to experience breakdown of isolating mechanisms than are groups that practice internal fertilization and pair during reproduction. Hubbs and Hubbs (1932) believe that interspecific hybrids in the Cyprinidae are, "due to the chance meeting of egg and sperm of distinct species." Groups in which there is brief pairing at the time of fertilization are more susceptible to mis-mating than are groups in which there is more or less permanent pairing and care of the progeny. As Mayr (1942, p. 262) puts it, in the latter case the animals have not only committed an original mistake but have apparently not corrected it afterward. The occurrence of natural interspecific hybrids has been reported in several groups of vertebrates, and these will be discussed below in relation to the isolating mechanisms that pertain in these groups.

The toads (*Bufo*) are representative of animals that form breeding swarms. Apparent hybrids have been described by A. P. Blair (1941a) between sympatric populations of toads classically treated as species and so considered by him. The principal mechanisms that prevent interbreeding between species of toads are: (1) partially different times of breeding, (2) partially different places of breeding, (3) different calls, although the effectiveness of call differences has not been demonstrated experimentally. There is considerable evidence that, once individuals are in close proximity, mating preference is of little importance as an isolating mechanism. Sexually excited males will clasp objects of appropriate size, even inanimate ones. When two species are breeding in the same pond, a female going to the call of a male of her own species might approach and be clasped by a male of another species. W. A. Thornton (unpublished data) has found cross-mated *Bufo woodhousii* and *Bufo valliceps* near Austin, Texas.

Fishes, with their wide range of breeding habits and consequent variation in complexes of isolating mechanisms, vary greatly in their potential susceptibility to interspecific crossing. Breeding swarms of pelagic spawners are presumably highly specific in their response to external stimuli, discharging eggs and sperm in response to these stimuli (see Mayr, 1942). Cross-fertilization could result from the chance mixing of gametes from different breeding swarms. The family Centrarchidae, a group in which natural hybridization had been widely studied (Hubbs and Hubbs, 1932; Bailey and Lagler, 1938; and others), appears to be rather susceptible to the breakdown of isolating mechanisms when the environment is disturbed by man. These fishes, "have a complex mating behavior, involving a nuptial gyration over the redd ('nest') prepared by the male" (Hubbs and Hubbs, 1932). However, "many females may visit one nest, and one female may visit many nests" (Breder, 1936). This last increases the chance for a mistake, where more than one species is spawning in the same locality. Noble (1934) and Breder (1936) have shown that in some species, at least, males will court inanimate objects irrespective of their general appearance when they are manipulated somewhat to resemble the actions of a female ready to spawn. This tends to indicate that differences in appearance of the females of different species

may be of little importance in identifying the females to the males. The final decision apparently rests with the females (see Breder, *op. cit.*), and it seems likely that coloration of the males may affect the decision of a female to approach or not approach the nest occupied by a male. Breder (1936) listed several factors that he considered favorable to the hybridization of species of centrarchids; these include: "the species are numerous; there is less geographic separation than usual; spawning occurs at about the same temperature threshold; spawning sites are limited and similar for most species; nests are exchanged among species." These observations by Breder would seem to indicate that the principal mechanism that prevents cross-fertilization in the sunfishes is the courtship pattern. Environmental factors, however, are indicated by others to be of considerable importance. Bailey and Lagler (1938) found all possible hybrid combinations between three sympatric species in an artificial lake where gravel bottom for nesting was scarce and in which all species were stunted due to overcrowding. Crowding of the populations and reduction of available spawning sites seem to be the chief factors responsible for hybridization in this case. Hubbs and Hubbs (1932) also mention the association between reduced nesting areas and species hybridization in small lakes. Hubbs and Hubbs (1933) and Bailey and Lagler (1938) believe that the sunfish hybrids are sterile. If this is the case, it is hybrid sterility that prevents gene transfer between the species populations. The other isolating mechanisms serve principally to reduce the wastage of gametes in the production of sterile hybrids.

Isolating mechanisms in a group of fishes that practices internal fertilization have been recently studied by Haskins and Haskins (1949). Species discrimination and mating preference were demonstrated experimentally in three sympatric species of the family Poeciliidae. Their evidence indicates tentatively that discrimination rests with the male, although, "marked sexual dimorphism exists on the part of the male in form, coloration, and courtship pattern."

Permanent pairing, at least during the breeding season, and care of the young by both sexes is common among African species of the family Cichlidae (Kosswig, 1947). This is a reproductive pattern that should operate strongly against interbreeding of species populations, and Kosswig believes that it has, "been able to play an important part in the acceleration of species formation *in loco* in the Cichlidae of the great East African lakes."

Mice of the genus *Peromyscus* show patterns of reproductive behavior and complexes of isolating mechanisms that may be of widespread occurrence in the mammals. In the two species of *Peromyscus* that have been studied, the mice form more or less permanent pairs that generally exist so long as both mice remain alive (Howard, 1949; W. F. Blair, 1951). Care of the young by the male as well as by the female has been reported from this genus (Horner, 1947) and from a related genus, *Baiomys*, (W. F. Blair, 1944a). Mating preference, shown experimentally between *Peromyscus maniculatus* and *Peromyscus polionotus* by Blair and Howard (1944), is probably an isolating mechanism of major importance in this group. Ecological isolation also appears to be important.

Only one interspecific hybrid combination has been reported from nature in *Peromyscus*. Four presumed hybrids between *Peromyscus leucopus* and *Peromyscus gossypinus* were reported from Alabama by Howell (1921). These species cross fairly readily in the laboratory and produce fertile F₁ hybrids of both sexes. The two populations are partially separated in nature by the preference of *leucopus* for higher, drier forests and the preference of *gossypinus* for lower, moister forests. Two other sympatric species, *truei* and *nasutus*, have been crossed in the laboratory to produce fertile female and sterile male hybrids, but no recognizable hybrid between these species has been found in nature. Mating preference seems to be a strong isolating mechanism in both of these cases.

Birds are like mammals in that the young are cared for by one or both parents and in that permanent pairs are formed in many groups during the breeding season. Mayr (1942) points out that, "sympatric hybrids are formed primarily in genera in which copulation is not preceded by pair formation and an 'engagement period.'" "Hybrids occur much more rarely among pair-forming species of birds."

Chapin (1948) has recently reported presumed hybrids involving three sympatric species of paradise flycatchers in Africa due to loss of ecological isolation following clearing of the rain forest. If confirmed, this will be a remarkable case among the higher vertebrates, for these birds form pairs and both parents care for the young. The apparent dependence of these three populations on ecological barriers as isolating mechanisms strongly suggests that these populations are in a much less advanced stage of speciation than their morphological differentiation would seem to indicate.

Why Are Species Hybrids Scarce?

Although natural hybrids between sympatric species have been reported from many vertebrate groups, the discovery of such hybrids is a comparatively rare event. Hybrids between sympatric species do appear to be much more rare in animals than in plants. Mayr (1942) reported that only two or three dozen hybrids are known from more than 100,000 bird of paradise skins exported from New Guinea. This low incidence of hybrids is significant in view of the fact that social patterns are more favorable to hybridization in this group than in most birds, for there is no pair formation and care of young by both parents in most of the species. Even in the fishes, from which species hybrids have been reported more frequently than from other groups, Hubbs, Hubbs and Johnson (1943) in analyzing natural hybridization in suckers (*Catostomidae*) estimated that in those parts of the stream systems where both parental species occur, "perhaps one sucker in 100 is an interspecific hybrid." Under conditions highly favorable to failure of isolating mechanisms, Bailey and Lagler (1938) found recognizable hybrids comprising as high as thirteen per cent. of centrarchid populations. The most striking evidence that natural species hybrids are rare in animals comes from the work with *Drosophila*. In this group, in which natural populations have been sampled more extensively than in probably any other group of

may be of little importance in identifying the females to the males. The final decision apparently rests with the females (see Breder, *op. cit.*), and it seems likely that coloration of the males may affect the decision of a female to approach or not approach the nest occupied by a male. Breder (1936) listed several factors that he considered favorable to the hybridization of species of centrarchids; these include: "the species are numerous; there is less geographic separation than usual; spawning occurs at about the same temperature threshold; spawning sites are limited and similar for most species; nests are exchanged among species." These observations by Breder would seem to indicate that the principal mechanism that prevents cross-fertilization in the sunfishes is the courtship pattern. Environmental factors, however, are indicated by others to be of considerable importance. Bailey and Lagler (1938) found all possible hybrid combinations between three sympatric species in an artificial lake where gravel bottom for nesting was scarce and in which all species were stunted due to overcrowding. Crowding of the populations and reduction of available spawning sites seem to be the chief factors responsible for hybridization in this case. Hubbs and Hubbs (1932) also mention the association between reduced nesting areas and species hybridization in small lakes. Hubbs and Hubbs (1933) and Bailey and Lagler (1938) believe that the sunfish hybrids are sterile. If this is the case, it is hybrid sterility that prevents gene transfer between the species populations. The other isolating mechanisms serve principally to reduce the wastage of gametes in the production of sterile hybrids.

Isolating mechanisms in a group of fishes that practices internal fertilization have been recently studied by Haskins and Haskins (1949). Species discrimination and mating preference were demonstrated experimentally in three sympatric species of the family Poeciliidae. Their evidence indicates tentatively that discrimination rests with the male, although, "marked sexual dimorphism exists on the part of the male in form, coloration, and courtship pattern."

Permanent pairing, at least during the breeding season, and care of the young by both sexes is common among African species of the family Cichlidae (Kosswig, 1947). This is a reproductive pattern that should operate strongly against interbreeding of species populations, and Kosswig believes that it has, "been able to play an important part in the acceleration of species formation *in loco* in the Cichlidae of the great East African lakes."

Mice of the genus *Peromyscus* show patterns of reproductive behavior and complexes of isolating mechanisms that may be of widespread occurrence in the mammals. In the two species of *Peromyscus* that have been studied, the mice form more or less permanent pairs that generally exist so long as both mice remain alive (Howard, 1949; W. F. Blair, 1951). Care of the young by the male as well as by the female has been reported from this genus (Horner, 1947) and from a related genus, *Baiomys*, (W. F. Blair, 1944a). Mating preference, shown experimentally between *Peromyscus maniculatus* and *Peromyscus polionotus* by Blair and Howard (1944), is probably an isolating mechanism of major importance in this group. Ecological isolation also appears to be important.

Only one interspecific hybrid combination has been reported from nature in *Peromyscus*. Four presumed hybrids between *Peromyscus leucopus* and *Peromyscus gossypinus* were reported from Alabama by Howell (1921). These species cross fairly readily in the laboratory and produce fertile F_1 hybrids of both sexes. The two populations are partially separated in nature by the preference of *leucopus* for higher, drier forests and the preference of *gossypinus* for lower, moister forests. Two other sympatric species, *truei* and *nasutus*, have been crossed in the laboratory to produce fertile female and sterile male hybrids, but no recognizable hybrid between these species has been found in nature. Mating preference seems to be a strong isolating mechanism in both of these cases.

Birds are like mammals in that the young are cared for by one or both parents and in that permanent pairs are formed in many groups during the breeding season. Mayr (1942) points out that, "sympatric hybrids are formed primarily in genera in which copulation is not preceded by pair formation and an 'engagement period.'" "Hybrids occur much more rarely among pair-forming species of birds."

Chapin (1948) has recently reported presumed hybrids involving three sympatric species of paradise flycatchers in Africa due to loss of ecological isolation following clearing of the rain forest. If confirmed, this will be a remarkable case among the higher vertebrates, for these birds form pairs and both parents care for the young. The apparent dependence of these three populations on ecological barriers as isolating mechanisms strongly suggests that these populations are in a much less advanced stage of speciation than their morphological differentiation would seem to indicate.

Why Are Species Hybrids Scarce?

Although natural hybrids between sympatric species have been reported from many vertebrate groups, the discovery of such hybrids is a comparatively rare event. Hybrids between sympatric species do appear to be much more rare in animals than in plants. Mayr (1942) reported that only two or three dozen hybrids are known from more than 100,000 bird of paradise skins exported from New Guinea. This low incidence of hybrids is significant in view of the fact that social patterns are more favorable to hybridization in this group than in most birds, for there is no pair formation and care of young by both parents in most of the species. Even in the fishes, from which species hybrids have been reported more frequently than from other groups, Hubbs, Hubbs and Johnson (1943) in analyzing natural hybridization in suckers (*Catostomidae*) estimated that in those parts of the stream systems where both parental species occur, "perhaps one sucker in 100 is an interspecific hybrid." Under conditions highly favorable to failure of isolating mechanisms, Bailey and Lagler (1938) found recognizable hybrids comprising as high as thirteen per cent. of centrarchid populations. The most striking evidence that natural species hybrids are rare in animals comes from the work with *Drosophila*. In this group, in which natural populations have been sampled more extensively than in probably any other group of

animals and in which many species have been crossed in the laboratory, Patterson (1947) reported that hybrids between only one species pair had been found in nature.

The scarcity of recognized natural hybrids between vertebrate species might be explained in several ways: (1) once isolating mechanisms have developed, they are so effective that the species do not hybridize in nature, or they hybridize so infrequently that hybrids are exceptionally rare, (2) too little work has been done to reveal the extent of natural hybridization, (3) hybrids are produced but are generally unrecognizable as such.

Anderson (1948) favoring the third explanation above holds that, "when hybrids do occur they usually perpetuate themselves, if at all, in backcrosses to one or the other parental species, and the mongrel nature of their descendants is not apparent to the ordinary biologist." He also emphasizes the fact, apparent also from the study of vertebrate hybrids, that natural hybrids are usually found in association with man-made disturbances of the environment. Anderson's explanation is that hybrids are generally able to survive only where the habitat has been "hybridized" due to human disturbance. This theory cannot be proved or disproved on what we now know about the survival value of different genetic combinations in nature in the vertebrates. An equally tenable hypothesis would be that hybrids are found in disturbed environments because the disturbance has lowered ecological barriers to interbreeding.

What is the Effect of Sympatric Hybridization?

The amount of gene transfer between sympatric, vertebrate species is difficult to evaluate. It is my opinion that the rare hybridization of sympatric species in nature accounts for very little transfer of genes from one species to another. The strongest argument comes from the fact that the morphological differences between most sympatric species are clear-cut ones. In the cases where hybrids have been reported, the parental species maintain their identity even though local, hybrid swarms may be formed. We recognize, of course, that the possibility of gene transfer, or introgression, exists in any case in which interfertile species hybridize. We know virtually nothing about the social relationships of natural hybrids to the parental populations, or about the survival value of hybrid genotypes in nature. The possible behavior of hybrids toward the parental species has been tested in one case in *Peromyscus* (Blair and Howard, 1944). The species *maniculatus* and *polionotus*, which are geographically isolated and which show strong mating preference in the laboratory, exhibit marked heterosis in the F_1 . The hybrids pair indiscriminately with the parent *maniculatus* but show sexual isolation from the *polionotus*. In the hypothetical event of future overlap of range and of the breakdown of the mating preference bar to interbreeding, there could presumably be a transfer of *polionotus* genes into *maniculatus*, with little or no counter flow of *maniculatus* genes into *polionotus*. This assumes, of course, that the hybrids would survive in nature to sexual maturity.

If interspecific hybridization is as common in animals as Anderson (1949) believes it to be in plants, the mechanisms of reproductive isolation between

species should tend to disappear. Most biologists agree that these mechanisms are genetic in origin. Interbreeding would tend to dissipate such isolating mechanisms between the parent species as mating preference and courtship pattern even if the original hybrids tended to perpetuate themselves principally in backcrosses to the parent species, as suggested by Anderson.

INTERBREEDING OF INTRASPECIFIC POPULATIONS

Morphologically differentiated populations exist within the limits of a single species in all vertebrate groups. In fact, the polytypic species has been recognized as such for many years by the students of some vertebrate groups. Recognition of the fact that the interbreeding (or hybridization if you prefer), as we see it in nature, of different infraspecific populations may come about in different ways has been a rather recent development. Mayr (1942, p. 99) distinguishes between what he calls primary intergradation and secondary intergradation in polytypic species populations. In the case of primary intergradation, contiguous, continuously distributed populations have differentiated morphologically into geographical races, which are connected by so-called zones of intergradation in which there is a steepening of character gradients. In the case of secondary intergradation, populations that have been separated geographically in the past without developing effective reproductive isolation have reestablished contact and now interbreed in the zone of overlap. There is a steepening of the character gradient in this case, also, in the zone of hybridization. The interbreeding of morphologically unlike populations and consequent gene exchange in both of these cases is called introgressive hybridization by Anderson (1949). I believe the general tendency in the literature is to call the first case introgressive hybridization of subspecies. The second case is frequently called introgressive hybridization of species because of the conventional taxonomic practice of treating differentiated, allopatric populations as full species. This is an artificial distinction if we accept the modern, biological definition of the species, because the populations showing secondary intergradation are demonstrating that they have not developed effective isolating mechanisms. A great majority of the cases of so-called introgressive hybridization in both plants and animals appear to be cases of either primary or secondary intergradation. For this reason, it is useful to review the dynamics of natural populations in respect to the origin of both of these types of interbreeding of populations.

Primary Intergradation

The differentiation into geographic races within a continuously distributed population apparently results from the combined actions of the forces of: (1) mutation, (2) selection, and (3) retarded gene flow due to the existence of ecological barriers (see Wright, 1931, ff.). The evidence from *Peromyscus* has been summarized by Dice (1940a) and by W. F. Blair (1950). Selection seems to be the most important single factor affecting the dispersal of mutant

genes in the species population, and consequently affecting the gene complexes of the infraspecific populations, although population structure and pattern of distribution also have important effects. It is not too much of a generalization to say that the local gene complexes within a species population are a reflection of the environmental complexes within the range of the species. Where the environment changes gradually, there is gradual change in the morphological characters of the species to produce what the taxonomist would call a wide zone of intergradation. If the change in environment is gradual from one end of the species range to the other, morphologically quite different populations at the extremes of the species range may be connected by gradual clines in morphological characters, without any abrupt break in the character gradient. Where the environment changes abruptly, there may be an abrupt change in characters of the species. In this case, two well differentiated populations may exist contiguously and be connected by morphologically intermediate populations in the zone of abrupt environmental change.

Zones of primary intergradation have been studied more intensively in *Peromyscus* than in probably any other group of vertebrates. A broad zone of intergradation between *Peromyscus maniculatus bairdii* and *Peromyscus m. osgoodi* across North Dakota was found by Dice (1940b) to correspond to a gradual increase in elevation, decrease in amount of precipitation, increase in the arid character of the vegetation and increase in paleness of the soil. A narrow zone of intergradation between the well-marked subspecies *Peromyscus polionotus polionotus* and *Peromyscus p. albifrons* in western Florida has been intensively studied (Sumner, 1929a, 1929b, 1932). The *albifrons* occurs on pale-colored sands of coastal beaches and is replaced by *polionotus* on the dark-colored soils of the interior. The two forms intergrade in a zone only a few miles wide, inland from the coast. Much of the color difference between the two populations is due to the action of a single pair of alleles affecting coat pattern (W. F. Blair, 1944b). The dominant gene, acting to restrict distribution of pigment, and affected by a complex of minor modifying genes, predominates in the *albifrons* population. The *polionotus* population is almost or completely homozygous for the recessive allele that produces "normal" pigmentation. Selection in favor of adaptive color types on the very differently colored beach and inland soils can account for the origin and continued existence of these color populations. Haldane (1948) using our data, and making certain necessary assumptions, some of which unfortunately are not justified, calculated that the intensity of selection in the *Peromyscus polionotus* populations would have to be only 0.1 per cent. to account for the situation discussed above. It seems likely that the intensity of selection on the two soil types is considerably higher than this, and Haldane (*op. cit.*) believes it is probable that, "the light varieties were formed under the action of much more intense selection on the beaches." In this case, and in others studied in *Peromyscus*, environmental selection appears to be the most important factor maintaining the geographic races. A change in the environment in any part of the species range might favor

extension of the range of one adaptive gene complex at the expense of another. Addition of organic material to darken the beach soils would favor extension of the polionotus type onto the beaches. On the other hand, an environmental change might change the conditions of selection so much that the characters of the local race would be changed simply due to the differential survival of mutants and to the shifting of existing, local gene frequencies. This change could come without any acceleration of gene flow from contiguous populations. Additional darkening of the inland soils would shift selection there to favor survival of the darkest-colored polionotus, but it would maintain or intensify selection against the albifrons type.

The physiological races of *Rana pipiens* reported by Moore (1949) also show adaptive differentiation of sub-populations within the species population. Northern populations differ from southern ones in such adaptive characters as embryonic temperature tolerance, rate of development, effect of temperature change on rate of development, egg size, and form of egg mass. These are adaptations to particular environments just as much as pelage colors and tail length are adaptations to particular environments in *Peromyscus*. The adaptations in *Rana* are principally adaptations to the physical environment, while those in *Peromyscus* are adaptations to the sum of the physical and biological environment. Another case has been analyzed by Heuts (1947) in the European stickleback (*Gasterosteus aculeatus*). Two sharply defined, physiologically adapted populations occur in Belgium, one in fresh water and the other in salt. The two populations differ in such morphological characters as plate number and body size and in physiological traits, the most important of which concerns chlorine elimination from the blood. The change from one type to the other is rapid, where the water changes rapidly from fresh to salty and brackish. Gene flow between the two populations is limited by selection against the adults and even more strongly against the eggs.

That the genotypes of infraspecific populations are principally the result of environmental pressures is probably more clearly recognized by botanists than by zoologists. The work of Turesson (1922), Clausen, Keck and Hiesey (1941, ff.), and Gregor (1939, ff.) goes beyond anything yet accomplished by the zoologists in demonstrating the adaptive character of geographic sub-populations of species.

If, as we believe, geographic races are molded by their environments—ecological trends of Dice (1940a)—the gene complexes of these races must shift continuously in response to local shifts in environmental conditions. Gershenson (1945) found cyclic, seasonal shifts in the frequency of black and agouti color types in the hamster (*Cricetus cricetus*). Dobzhansky (1943) has demonstrated such cyclic shifts in gene arrangements in natural populations of *Drosophila pseudoobscura*, and Dobzhansky (1947) and Dobzhansky and Levene (1948) have shown these shifts to be caused by natural selection. These findings all bear out the more indirect evidence from *Peromyscus* that the gene complexes of natural populations are molded by the environment, and they suggest a sensitive relationship between gene frequency and

environment. Wright (1949) points out that, "a continual, kaleidoscopic shifting of the statistical characters is to be expected within any species that occupies, not too densely, a reasonably large range."

There is some objection to the use of the specialized term introgressive hybridization in reference to the long-recognized phenomenon of gene flow between infraspecific populations. The idea of the progression of one form into the other is implicit in the term introgressive hybridization if we interpret correctly the usage and implications of the term by Anderson (1949), Heiser (1949), Woodson (1947) and others. The idea of the movement of one form into another is not consistent with the concept that geographic races exist as environmentally controlled gene complexes. Just because two morphologically dissimilar forms interbreed freely in a zone of contact does not mean that the genes of either population will be distributed freely throughout the range of the other. The rate of gene flow between sub-populations of a species is controlled by many factors, including principally: (1) population density and structure, (2) pattern of distribution, and (3) rate of selection. Gene dispersal from one geographic race into another will be controlled largely by environmental selection. In other words, an equilibrium must exist between gene flow and selective elimination of immigrants to permit the origin and perpetuation of geographic races within a species population. A polytypic species nevertheless evolves as a single system, as Dobzhansky (1949a) points out, although its geographic races are adapted to the environments of their respective territories. Any gene or gene arrangement possessed by the species theoretically may be dispersed to any part of the species range. Whether it is so dispersed or not depends on ecological factors.

The rate of differentiation of adjacent sub-populations will depend, of course, on the level at which equilibrium between immigration and selection is reached. A case in point is that of the black-colored races of rodents on the Tularosa Malpais and other lava flows in the Southwest (see Benson, 1933; W. F. Blair, 1943b, 1947; Dice, 1939; Dice and Blossom, 1937). One species, *Peromyscus eremicus*, is represented on the Malpais by a highly variable population in which some individuals are highly melanistic and others are nearly or quite as pale as the populations occupying the limestone rocks of the nearby hills. A reasonable, although hypothetical, history of the evolution of this color race would be as follows. The lava flowed down the Tularosa Basin, possibly as recently as 10,000 years ago to cover an area about forty miles long and from one to six miles wide. As vegetation invaded the lava bed there was also an invasion of pale-colored *eremicus* from the limestone hills. Existence was probably precarious for the pale-colored mice on the dark lava rock, and high immigration pressure from the surrounding populations may have been necessary to maintain even a sparse population on the lava. Mutations toward a melanistic coat color presumably occurred in the lava-bed populations at the same rate as they had occurred in the parent population. Such mutations were now of distinct advantage to their possessors. The melanistic mutants showed a higher survival rate and

consequently a higher reproductive rate than the non-mutants, whereas they had been selected against in the parent populations. A continued high rate of immigration has prevented the development of a uniformly dark-colored population on the lava bed. If this has been the history of the evolution of the dark-colored population, then differentiation has taken place against the pressure of continued immigration. The present frequencies of the color genes in the lava-bed population are apparently a reflection of the equilibrium between selection pressure and immigration pressure. We cannot discount the possibility that the genes for paleness of pelage color have existed in the lava-bed populations since the lava bed was colonized. Immigration pressure is indicated, however, as a contributing cause of the variability by the fact that the lava-bed population of *eremicus* is less isolated ecologically than are other rodent populations that show less variability on the lava bed. We cannot overlook the possibility that selection on the lava bed favors heterozygotes in a manner similar to that described by Dobzhansky (1948) from *Drosophila*. The differences between the dark and pale mice are apparently due to multiple alleles. Selection in favor of heterozygotes would not result in the elimination of either extreme from the population.

The effects of potential gene flow and of selection on adaptive differentiation of infraspecific populations has been studied in *Peromyscus maniculatus blandus* in southern New Mexico (W. F. Blair, 1947b). Allelic genes with major effects on pelage color showed adaptive differentiation in frequency (75 per cent. recessive gray on gray soil and 43 per cent. gray on red soil) at stations eighteen miles apart in a continuously distributed population. There was no differentiation in the frequency of these color genes on differently colored soils at stations four miles apart in a continuously distributed population. Populations twenty miles apart, on similarly colored soils and separated by a strong ecological barrier, showed no differentiation in the frequency of these color genes. Both ecological isolation and environmental selection affect the differentiation of these local populations. Isolation by distance, as little as eighteen miles in this case, coupled with selection, permits adaptive differentiation. Parallel selection in similar environments inhibits differentiation even when there is reduced gene flow between the populations.

Our second objection to the use of the term introgressive hybridization in reference to gene flow between infraspecific populations is that previous geographic discontinuity of the populations is implied, or even postulated. By emphasizing the presumed merging of what are assumed to be previously discrete populations, the proponents of introgressive hybridization tend to overlook the fact that zones of intergradation between subspecies are maintained by the balance between gene flow and selective elimination of immigrants into the respective populations. A case in point is Woodson's (1947) study of *Asclepias tuberosa*. His distribution map for the species shows an excellent example of what I would call primary intergradation of three geographic races. I doubt that the origin of these geographic races has been very different from that discussed above for geographic races of mammals

connected by zones of primary intergradation. Woodson, on the other hand, postulates, "origin in isolation on the Paleozoic and early Mesozoic land masses Ozarkia, Appalachia, and Orange Island." There is no proof in his data for the conclusion that a certain leaf modification, "because of certain associated selective advantages, appears to be supplanting the ancestral race." We might just as well say that *Peromyscus maniculatus osgoodi* is swamping *Peromyscus m. bairdii* because the change from one to the other takes place over a wide area and because *osgoodi* is a larger and therefore presumably a more vigorous mouse.

Secondary Intergradation

Secondary intergradation, as stated earlier, has a history entirely different from primary intergradation, although it is not always possible to determine which phenomenon is involved when examining specific cases. As stated earlier, speciation usually seems to be initiated by the spatial isolation of a part of a previously interbreeding population. The isolated populations gradually come to differ in morphological and in other characters for reasons that need no review here. These populations will tend to acquire differences that will prevent their interbreeding if the separate populations should ever come to occupy the same region. That isolating mechanisms develop gradually is indicated by the fact that several different mechanisms usually act to prevent interbreeding of sympatric species (table 1). Spatial isolation is, of course, an isolating mechanism of great importance in itself. Its weakness lies in the fact that its effects can be cancelled by any shift in range that would bring the previously isolated populations into juxtaposition. If such a shift in range comes before the populations have acquired intrinsic isolating mechanisms, the two populations will interbreed in a zone of secondary intergradation, or one population might theoretically swamp the other.

The shifting of populations, both the spatial isolation of parts of previously interbreeding populations and the reestablishment of contact by previously isolated populations, apparently results principally from major, regional changes in environment. Present day distribution of species, of speciating allopatric populations, and of zones of secondary intergradation between previously separate populations must be interpreted in relation to Pleistocene climatic shifts. An excellent discussion of the relationship between present distribution of both plants and animals and Pleistocene climates is given by Deevey (1949), who takes the rather extreme position that, "nearly all well-studied cases of subspeciation and speciation point to the Pleistocene as the time of . . . previous isolation, and the occurrence of closely related forms in the same area is therefore attributed to post-Pleistocene alterations in geography and biogeography."

Isolation of fragments of warm-climate populations in Florida and Mexico was one of the major biogeographic results on this continent of the southward shift of cold during the Pleistocene glacial stages. The evidence from both plants and animals is well summarized by Deevey (*op. cit.*). The

fragmentation of bird populations in other glacial-stage refuges on this continent has been discussed by Rand (1948).

If the rates of evolution and of spread are different in different species, the population pairs formed by Pleistocene isolation in Florida and Mexico should vary greatly in their stage of speciation. We should expect to find: (1) isolated allopatric populations that have shown little morphological differentiation, (2) isolated, allopatric populations that have differentiated morphologically but have not developed intrinsic isolating mechanisms, (3) allopatric populations that have differentiated morphologically and have developed isolating mechanisms, (4) populations that are now sympatric due to post-glacial spread after effective isolating mechanisms were developed, and (5) populations that underwent a post-glacial spread and established contact before they had developed effective isolating mechanisms. In this last situation we would expect to find secondary intergradation between the previously isolated populations.

East-west populations attributable to Pleistocene isolation in Florida and Mexico are common among the vertebrates (table 2), but in most cases our information about the biological differentiation as opposed to strictly morphological differentiation is sadly lacking. A representative case in which there seems to have been little morphological differentiation of the still-isolated populations is that of the gopher frogs, *Rana capito* of Florida and the Atlantic coastal plain and *Rana areolata* of the Mississippi Valley west into Kansas, Oklahoma, and Texas. An apparently disjunct population on the coast of Mississippi and Alabama known as *Rana sevosia* will possibly be found to be continuous with the Florida *capito* when western Florida has been adequately explored. A remarkably similar example is found in the atrox group of rattlesnakes (see Gloyd, 1940). The population known taxonomically as the species *adamanteus* has spread from its presumed Pleistocene refuge in Florida northward along the Atlantic coast and westward along the Gulf coast to the Mississippi Delta. The western population, treated taxonomically as the species *atrox*, extends westward from the border of the pine-oak forest in Texas to California. A third population, the species *ruber*, presumably originated under Pleistocene isolation in Baja California, overlaps the range of *atrox* in southern California without any apparent interbreeding. Numerous other examples could be cited to show that population fragments isolated in Florida and the Southwest have not yet reestablished contact. An additional example in the tree frogs is that of *Pseudacris ornata* and *Pseudacris streckeri* with a distributional relationship very similar to that discussed above. It is unknown whether the species pairs mentioned above have developed effective isolating mechanisms that would prevent their interbreeding when and if they ever come to have overlapping ranges. In one case at least, that of *Peromyscus maniculatus* and *Peromyscus polionotus*, the allopatric populations have acquired mating preferences that should prevent interbreeding in case of overlap of range (Blair and Howard, 1944).

In a good many cases, the previously isolated populations have spread until there is overlap of range, with the two populations maintaining their

TABLE 2

PRESENT RELATIONSHIPS OF SOME NORTH AMERICAN AMPHIBIAN, REPTILE AND MAMMAL POPULATIONS THAT WERE PRESUMABLY FRAGMENTED DURING PLEISTOCENE GLACIAL STAGES

Populations		Present relationships
Eastern	Western	
<i>Rana capito-sevosa</i>	<i>Rana areolata</i>	Spatially isolated
<i>Pseudacris ornata</i>	<i>Pseudacris streckeri</i>	Spatially isolated
<i>Microhyla carolinensis</i>	<i>Microhyla olivacea</i>	Overlap; reproductive isolation
<i>Pseudacris nigrita</i>	<i>Pseudacris clarkii</i>	Overlap; reproductive isolation
<i>Bufo w. fowleri</i>	<i>Bufo w. woodhousii</i>	Overlap; secondary intergradation
<i>Crotalus adamanteus</i>	<i>Crotalus atrox</i>	Spatially isolated
<i>Pituophis c. sayi</i>	<i>Pituophis c. catenifer</i>	Overlap; secondary intergradation
<i>Peromyscus polionotus</i>	<i>Peromyscus maniculatus</i>	Spatially isolated; reproductive isolation
<i>Peromyscus gossypinus</i>	<i>Peromyscus leucopus</i>	Overlap; reproductive isolation
<i>Neotoma floridana</i>	<i>Neotoma micropus</i>	Meet, do not interbreed

distinctness in the zone of overlap. The *Peromyscus leucopus*-*P. gossypinus* pair is a good example in mammals. The ranges of the eastern *Neotoma floridana* and the western *Neotoma micropus* meet in central Texas. The ranges of these packrats apparently do not overlap, nor do the two populations appear to interbreed. Among the amphibians, the eastern *Microhyla carolinensis* and the western *Microhyla olivacea* overlap ranges in eastern Texas and Oklahoma. In eastern Texas, the two populations have different calls and are somewhat separated by different ecological preferences, although both have been found breeding in the same pond. A. P. Blair (1950) has reported similar behavior of the two populations in eastern Oklahoma. Hecht and Matalas (1946) claim to have found intergrades between the two populations at one locality in southeastern Oklahoma and at one locality in southern Texas. If true, this would indicate that the isolating mechanisms between the two populations are still weak. The eastern population of the widely distributed *Pseudacris nigrita* meets the range of *Pseudacris clarkii* in eastern Texas, Oklahoma, and Kansas. In Texas, at least, these behave as good species, having different calls and showing no evidence of interbreeding in their zone of overlap. In the case of all of these population pairs, the circumstantial evidence indicates that an effective complex of isolating mechanisms was developed either during the period of isolation or possibly, as Dobzhansky (1949b) suggests, through selection when the two populations reestablished contact.

Interbreeding of the previously separate populations is to be expected in the case of the populations that reestablished geographical contact before developing effective isolating mechanisms. Well-authenticated cases of secondary intergradation are comparatively rare in North American verte-

brates. There are probably two major reasons for this scarcity: (1) the interval between glacial-stage splitting of populations and subsequent spread was sufficiently long to allow specific differentiation (that is, acquisition of isolating mechanisms) as evidenced by the cases mentioned earlier above, (2) cases of secondary intergradation often cannot be distinguished from primary intergradation.

Cases of presumed secondary intergradation in several groups of European forms, following post-glacial spread, have been summarized by Mayr (1942). A few cases have been reported from North America, but some of these may be cases of primary intergradation. The frequently cited case of the flickers, the eastern *Colaptes auratus* and the western *Colaptes cafer*, which interbreed in a wide zone from Texas to British Columbia might be interpreted as either primary or secondary intergradation. Another frequently cited case, that of the bullsnakes (*Pituophis catenifer* and *Pituophis sayi*) now regarded as a single polytypic species could be a case of either primary or secondary intergradation.

At least one of the cases called species hybridization of toads by A. P. Blair (1941a) appears to represent a secondary contact and partial fusion of previously isolated populations. The eastern *Bufo fowleri* and the western *Bufo woodhousii* meet in eastern Oklahoma and Texas. In Oklahoma, "where the east-west topographic change is abrupt, as the transition from Ozark Hills to Osage Plain, the ranges of the two species meet, but few or no intermediates are found. Where change is more gradual (LaMine and Yuba) intermediate populations are found" (*idem*). The grassland-inhabiting woodhousii probably spread northward and eastward from a Pleistocene refuge in Mexico, following the retreat of the Wisconsin glaciers. The present contact permits interchange of genes along the line of secondary intergradation. There seems no reason to believe that the penetration of the genes of one population into the other will be any greater than that between two forms that show primary intergradation. Each form is adapted to a particular environment, woodhousii to grassland and fowleri to the deciduous forest, and selective elimination of non-adapted genotypes should act to maintain the adaptive characters of the two populations and to limit intermediates to the zone of transition from grassland to deciduous forest.

In *Peromyscus maniculatus*, the intergradation between grassland and forest forms in western North America is probably of the secondary type. This is an old species, as evidenced by its wide distribution, by its large number of continental and insular subspecies, and by the fact that it has given rise to other species around the periphery of its range (see Osgood, 1909; W. F. Blair, 1950). Two major ecological types exist within the species, and each type includes numerous subspecies. One is a short-tailed, short-eared, small-footed grassland form, while the other is a long-tailed, large-eared, large-footed inhabitant of forests. The forest form has a roughly U-shaped distribution, extending northward through the Appalachians, westward across the Canadian forests and southward in the mountains of western North America. The grassland form ranges northward from Mexico

and occupies the central grasslands inside the U-shaped range of the forest populations. The two forms meet without interbreeding in Glacier Park, Montana (see Murie, 1933; Mayr, 1942). Clearing of the forest has permitted the grassland form to reach the range of the forest form in Michigan and New York (see Dice, 1931; Hooper, 1942; Hamilton, 1950). The two forms interbreed in some places along the eastern front of the Rocky Mountains and in other parts of western North America. Post-Pleistocene changes in the climate of central North America presumably forced the withdrawal of the forest from this area and permitted the northward spread of the grassland form from a refuge in Mexico.

Numerous cases of both primary and secondary intergradation are cited by A. H. Miller (1941) in the avian genus *Junco*. The populations of this genus are, in Miller's words, "almost without exception geographically complementary." In addition to well-marked forms showing primary intergradation, there seems to be a considerable number of previously isolated populations that have rejoined ranges before developing effective isolating mechanisms. Miller relies considerably on morphological differentiation in recognizing ten species among these geographically complementary populations. In spite of this taxonomic expedient, I would regard the interbreeding of many of these named populations as secondary intergradation of populations that were in incipient stages of speciation before reestablishing contact in their ranges.

Reestablishment of contact and subsequent secondary intergradation by previously isolated populations should reestablish a relationship similar to that existing between infraspecific populations showing primary intergradation. Each secondarily intergrading population will be adapted to its own environment, and the interchange of genes between such populations will be conditioned by the same factors of selection and of population structure that affect the interchange of genes between populations showing primary intergradation. The reunited populations will now evolve as a single system.

SUMMARY

Sympatric species are prevented from interbreeding by complexes of isolating mechanisms, and hybridization involves the breakdown of some of these mechanisms. Closely related species are frequently interfertile in the vertebrates, and continued segregation of related sympatric species is dependent on the effectiveness of the isolating mechanism complex. Hybrids between sympatric species of vertebrates are rare, and they usually occur where there has been a breakdown of ecological isolation. The amount of gene transfer between sympatric species of vertebrates is unknown, but it seems probable that it is negligible.

The origin and perpetuation of morphologically differentiated subpopulations—geographic races—are controlled by mutation, selection, and amount of gene flow. Local gene complexes are, in general, reflections of the local environmental complexes. An equilibrium between gene flow and

INTERBREEDING OF NATURAL POPULATIONS OF VERTEBRATES 27

selective elimination of immigrants must exist to permit the origin and continued existence of geographic races. The polytypic species evolves as a single system because of gene exchange, but each race is adapted to its own environment.

Fragments of species populations that have been spatially isolated, as by Pleistocene climatic shifts, may rejoin ranges before an effective complex of isolating mechanisms has been developed. Under such circumstances, the previously isolated populations will show secondary intergradation along the line of contact. The interchange of genes between secondarily interbreeding populations will be conditioned by the same factors of selection and population structure that affect the exchange of genes between geographic races that have not been spatially isolated. The reunited populations will now evolve as a single system. Populations of vertebrates showing all stages of incipient speciation, following Pleistocene fragmentation of ancestral populations, occur in North America.

LITERATURE CITED

- Anderson, Edgar, 1948, Hybridization of the habitat. *Evolution* 2: 1-9.
 1949, *Introgressive Hybridization*. 109 pp. New York: Wiley.
- Anderson, Edgar and Leslie Hubricht, 1938, The evidence for introgressive hybridization. *Amer. J. Bot.* 25: 396-402.
- Bailey, Reeve M. and Karl F. Lagler, 1938, An analysis of hybridization in a population of stunted sunfishes in New York. *Pap. Mich. Acad. Sci., Arts, and Letters* 23 (1937): 577-606.
- Benson, S. B., 1933, Concealing coloration among some desert rodents of the southwestern United States. *Univ. Calif. Publ. Zool.* 40: 1-70.
- Blair, Albert P., 1941a, Variation, isolation mechanisms, and hybridization in certain toads. *Genetics* 26: 398-417.
 1941b, Isolating mechanisms in tree frogs. *Proc. Nat. Acad. Sci.* 27: 14-17.
 1950, Note on Oklahoma microhylid frogs. *Copeia* 1950: 152.
- Blair, W. Frank, 1943a, Criteria for species and their subspecies from the point of view of genetics. *Annals New York Acad. Sci.* 44: 179-188.
 1943b, Ecological distribution of mammals in the Tularosa Basin, New Mexico. *Contr. Lab. Vert. Biol.* 20: 1-24.
 1944a, Observations on the life history of *Baiomys taylori subater*. *J. Mammal.* 22: 378-383.
 1944b, Inheritance of the white-cheek character in mice of the genus *Peromyscus*. *Contr. Lab. Vert. Biol.* 25: 1-7.
 1947a, Variation in shade of pelage of local populations of the cactus-mouse (*Peromyscus eremicus*) in the Tularosa Basin and adjacent areas of southern New Mexico. *Contr. Lab. Vert. Biol.* 37: 1-7.
 1947b, Estimated frequencies of the buff and gray genes (*G,g*) in adjacent populations of deer-mice (*Peromyscus maniculatus blandus*) living on soils of different colors. *Contr. Lab. Vert. Biol.* 36: 1-16.
 1950, Ecological factors in speciation of *Peromyscus*. *Evolution* 4: 253-275.
 1951, Population structure, social behavior, and environmental relations in a natural population of the beach mouse (*Peromyscus polionotus leucocephalus*). *Contr. Lab. Vert. Biol.* (in press).
- Blair, W. Frank and Walter E. Howard, 1944, Experimental evidence of sexual isolation between three forms of mice of the cenospecies *Peromyscus maniculatus*. *Contr. Lab. Vert. Biol.* 26: 1-19.
- Breder, C. M., 1936, The reproductive habits of the North American sunfishes (family Centrarchidae). *Zoologica* 21: 1-48.

- Chapin, James P., 1948, Variation and hybridization among the paradise flycatchers of Africa. *Evolution* 2: 111-126.
- Clausen, Jens, David D. Keck, and William M. Hiesey, 1941, Regional differentiation in plant species. *Amer. Nat.* 75: 231-250.
- 1947, Heredity of geographically and ecologically isolated races. *Amer. Nat.* 81: 114-133.
- Deevey, Edward S., Jr., 1949, Biogeography of the Pleistocene. *Bull. Geol. Soc. Amer.* 60: 1315-1416.
- Dice, Lee R., 1931, The occurrence of two subspecies of the same species in the same area. *J. Mammal.* 12: 210-213.
- 1933, Fertility relationships between some of the species and subspecies of mice in the genus *Peromyscus*. *J. Mammal.* 14: 298-305.
- 1937, Fertility relations in the *Peromyscus leucopus* group of mice. *Contr. Lab. Vert. Genetics* 4: 1-3.
- 1939, Variation in the cactus-mouse, *Peromyscus eremicus*. *Contr. Lab. Vert. Genetics* 8: 1-27.
- 1940a, Ecologic and genetic variability within species of *Peromyscus*. *Amer. Nat.* 74: 212-221.
- 1940b, Intergradation between two subspecies of deer-mouse (*Peromyscus maniculatus*) across North Dakota. *Contr. Lab. Vert. Genetics* 13: 1-14.
- Dice, Lee R. and Philip M. Blossom, 1937, Studies of mammalian ecology in southwestern North America with special attention to the colors of desert mammals. *Carnegie Inst. Wash. Publ.* 485: 1-129.
- Dice, Lee R. and Margaret Liebe, 1937, Partial infertility between two members of the truei group of mice. *Contr. Lab. Vert. Genetics* 5: 1-4.
- Dobzhansky, Th., 1943, Genetics of natural populations. IX. Temporal changes in the composition of populations of *Drosophila pseudoobscura*. *Genetics* 28: 162-186.
- 1947, Genetics of natural populations. XIV. A response of certain gene arrangements in the third chromosome of *Drosophila pseudoobscura* to natural selection. *Genetics* 32: 142-160.
- 1948, Genetics of natural populations. XVIII. Experiments on chromosomes of *Drosophila pseudoobscura* from different geographic regions. *Genetics* 33: 588-602.
- 1949a, Observations and experiments on natural selection in *Drosophila*. *Proc. Eighth Int. Cong. Genetics, Hereditas, Suppl. vol.*: 210-224.
- 1949b, On some of the problems of population genetics and evolution. *La Ricerca Scientifica* 19: 1-9.
- Dobzhansky, Th. and Howard Levene, 1948, Genetics of natural populations. XVII. Proof of operation of natural selection in wild populations of *Drosophila pseudoobscura*. *Genetics* 33: 537-547.
- Gershenson, S., 1945, Evolutionary studies on the distribution and dynamics of melanism in the hamster (*Cricetus cricetus* L.). *Genetics* 30: 207-251.
- Gloyd, Howard K., 1940, The rattlesnakes, genera *Sistrurus* and *Crotalus*. *Special Publ. Chicago Acad. Sci.* 4: 1-266.
- Gregor, J. W., 1939, Experimental taxonomy. IV. Population differentiation in North American and European sea plantains allied to *Plantago maritima* L. *New Phytologist* 38: 293-322.
- Haldane, J. B. S., 1948, The theory of a cline. *J. Genetics* 48: 277-284.
- Hamilton, W. J., 1950, The prairie deer mouse in New York and Pennsylvania. *J. Mammal.* 31: 100.
- Haskins, Caryl P. and Edna F. Haskins, 1949, The role of sexual selection as an isolating mechanism in three species of poeciliid fishes. *Evolution* 3: 160-169.

INTERBREEDING OF NATURAL POPULATIONS OF VERTEBRATES 29

- Hecht, Max K. and Bessie M. Matalas, 1946, A review of the middle North American roads of the genus *Microhyla*. Amer. Mus. Novitates 1315: 1-21.
- Heiser, Charles B., Jr., 1949, Natural hybridization with particular reference to introgression. Bot. Review 15: 645-687.
- Heuts, M. J., 1947, Experimental studies on adaptive evolution in *Gasterosteus aculeatus* L. Evolution 1: 89-102.
- Hooper, E. T., 1942, An effect on the *Peromyscus maniculatus* rassenkreis of land utilization in Michigan. J. Mammal. 23: 193-196.
- Horner, B. Elizabeth, 1947, Paternal care of young mice of the genus *Peromyscus*. J. Mammal. 28: 31-36.
- Howard, Walter E., 1949, Dispersal, amount of inbreeding, and longevity in a local population of prairie deer mice on the George Reserve, southern Michigan. Contr. Lab. Vert. Biol. 43: 1-50.
- Howell, Arthur H., 1921, A biological survey of Alabama. North Amer. Fauna 45: 1-88.
- Hubbs, Carl L. and Laura C. Hubbs, 1932, Experimental verification of natural hybridization between distinct genera of sunfishes. Pap. Mich. Acad. Sci., Arts, and Letters 15 (1931): 427-437.
- 1933, The increased growth, predominant maleness, and apparent infertility of hybrid sunfishes. Pap. Mich. Acad. Sci., Arts, and Letters 17 (1932): 613-641.
- Hubbs, Carl L., Laura C. Hubbs, and Raymond E. Johnson, 1943, Hybridization in nature between species of catostomid fishes. Contr. Lab. Vert. Biol. 22: 1-76.
- Kosswig, Curt, 1947, Selective mating as a factor for speciation in cichlid fishes of East African lakes. Nature 159: 604-605.
- Mainland, G. B., 1942, Genetic relationships in the *Drosophila funebris* group. Univ. Tex. Publ. 4228: 74-112.
- Mayr, Ernst, 1942, Systematics and the Origin of Species. 334 pp. New York: Columbia Univ. Press.
- 1947, Ecological factors in speciation. Evolution 1: 263-288.
- 1948, The bearing of the new systematics on genetical problems, the nature of species. Advances in Genetics 2: 205-237.
- 1949, Speciation (in Genetics, Paleontology, and Evolution). 281-298 Princeton: Princeton Univ. Press.
- Miller, Alden H., 1941, Speciation in the avian genus *Junco*. Univ. Calif. Publ. Zool. 44: 173-434.
- Miller, Robert R., 1948, The cyprinodont fishes of the Death Valley system of eastern California and southwestern Nevada. Misc. Publ. Univ. Mich. Mus. Zool. 68: 1-155.
- Moore, John A., 1946, Hybridization between *Rana palustris* and different geographical forms of *Rana pipiens*. Proc. Nat. Acad. Sci. 32: 209-212.
- 1949, Geographic variation of adaptive characters in *Rana pipiens* Schreber. Evolution 3: 1-24.
- Murie, Adolph, 1933, The ecological relationships of two subspecies of *Peromyscus* in the Glacier Park region, Montana. Occ. Pap. Univ. Mich. Mus. Zool. 270: 1-17.
- Noble, G. K., 1934, Sex recognition in the sunfish, *Eupomotis gibbosus* (Linné). Copeia 1934: 151-155.
- Osgood, Wilfred H., 1909, Revision of the mice of the American genus *Peromyscus*. North Amer. Fauna 28: 1-285.
- Patterson, J. T., 1942, Interspecific hybridization in the genus *Drosophila*. Univ. Tex. Publ. 4228: 7-15.
- 1947, Sexual isolation in the mulleri subgroup. Univ. Tex. Publ. 4720: 32-40.
- Rand, A. L., 1948, Glaciation, an isolating factor in speciation. Evolution 2: 314-321.

- Sumner, F. B., 1929a, An analysis of a concrete case of intergradation between two subspecies. *Proc. Nat. Acad. Sci.* 15: 110-120.
- 1929b, An analysis of a concrete case of intergradation between two subspecies. II. Additional data and interpretations. *Proc. Nat. Acad. Sci.* 15: 481-493.
- 1932, Genetic, distributional, and evolutionary studies of the subspecies of deer mice (*Peromyscus*). *Bibliog. Genetica* 9: 1-106.
- Trewavas, Ethelwynn, 1947, Speciation in cichlid fishes of East African lakes. *Nature* 160: 96-97.
- Turesson, G., 1922, The genotypical response of the plant species to the habitat. *Hereditas* 3: 341-347.
- Watson, Margaret Liebe, 1942, Hybridization experiments between *Peromyscus polionotus* and *Peromyscus maniculatus*. *J. Mammal.* 23: 315-316.
- Woodson, Robert E., Jr., 1947, Some dynamics of leaf variation in *Asclepias tuberosa*. *Ann. Missouri Bot. Garden* 34: 353-432.
- Wright, Sewall, 1931, Evolution in mendelian populations. *Genetics* 16: 97-159.
- 1949, Adaptation and selection (in *Genetics, Paleontology, and Evolution*). 365-389 Princeton: Princeton Univ. Press.

THE TAXONOMIC UNITS IN RELATION TO CYTOGENETICS
AND GENE ECOLOGY¹

R. RUGGLES GATES

Harvard University, Cambridge, Massachusetts

The history of the species question is one of the longest and most complicated in biology. I shall not attempt to recount it here, but I wish to deal mainly with the subject in its modern aspects, from a biological point of view. Having made extensive genetical-taxonomic investigations of the genus *Oenothera* over a period of many years (Gates, 1936) as well as briefer studies of many other plant genera, I should not be entirely unfitted for this task. There have been innumerable attempts to define species, but none of them have proved satisfactory or of universal application. Let me emphasize first that an inclusive definition of species which would apply to all groups of organisms is a will-o'-the-wisp, which would be of no value even if attainable. How can one expect to apply the same concept of species to, for example, bacteria and mammals or, let us say, to Cyanophyceae and flowering plants? To do so, considering only the vast range of differences in complexity of these organisms, implies an acrobatic effort in semantics, without finding any tangible common denominator except that in every group of organisms there are many different kinds. Moreover, the generic and specific differences appear to be no less marked in a group such as the Cyanophyceae, without sexual reproduction, than in the Rhodophyceae where sexual reproduction rules.

When we consider the multitude of ways in which new inheritable forms can arise, as disclosed by the modern work in cytogenetics and in experimental ecology, the futility of trying to comprise them all under one formula begins to appear. There is nevertheless one school of geneticists who have attempted to erect reproductive isolation as *the* criterion of species. This attitude, and the chaos to which it leads in animal species, has been criticized elsewhere (Gates, 1948). In short, it induces Dobzhansky, a leading exponent of this view, to be an ultra-splitter in one genus and an ultra-lumper in another. Dobzhansky (1946) describes a "new species" of *Drosophila*, *D. equinoxialis*, which will not cross with *D. willistoni* although there is a "virtual lack of morphological differences." There is no difference in the male external genitalia, the chromosomes are alike in both forms, but there is a mean difference in body size although the curves of

¹Invitation paper presented at a joint meeting of the four Sections, Genetics, Cytology, Experimental Taxonomy and Taxonomy of Phanerogams, at the VIIth International Botanical Congress, Stockholm, July, 1950.

size variation broadly overlap. Average individuals cannot be sorted into one "species" or the other; but because the reproductive isolation is virtually complete it is affirmed that there is "no reasonable doubt that these forms are full-fledged species rather than races of the same species." That morphologically identical forms are different species because they will not intercross, involves a contradiction in terms. The science of taxonomy is founded on the existence of visible differences by which forms can be classified. These *Drosophilas* are not full-fledged species, but if they continue in physiological isolation they may become so in ten thousand or fifty thousand years. But that is no justification for calling them species now. Microspecies would be a more appropriate name for them.

Drosophila persimilis and *D. pseudoobscura* are a similar pair of forms. Although partly sympatric and morphologically identical, reproductive isolation "appears to be complete in nature" through the cooperation of several isolating mechanisms. On this ground alone they are affirmed to be "unquestionably species." In opposing this view, Huxley (1940, p. 16) says, "When (Dobzhansky) tries to define the species-level as that stage in taxonomic differentiation after which fertile interbreeding is impossible, he goes far beyond the facts... it is impossible to insist on infertility as the sole criterion of species." One of the greatest defects of this view is that it ignores the aims and methods of taxonomy and seeks to make taxonomy subservient to a condition—sterility—in genetics, by raising it to the importance of a universal principle.

Muller (1942) is equally emphatic in stating (p. 96) that "in this genus (*Drosophila*) at least no sharp line can be drawn between sub-divisions of one rank, such as races or sub-species, and of another rank, such as species."

In a subsequent paper (Burla *et al*, 1949), Dobzhansky and his colleagues find that the four forms, *D. willistoni*, *D. paulistorum*, *D. equinoxialis* and *D. tropicalis*, are all sympatric in parts of Brazil. Although individuals of these microspecies could be distinguished with difficulty only in fresh material, even by those trained in *Drosophila* work, yet they are said to show varying degrees of sexual isolation and to be recognizable by the disc patterns in the chromosomes of the larval salivary glands. Only excessive zeal in applying the single criterion of intersterility would lead anyone to call them species rather than microspecies.

"Intersterility may in fact arise at any stage in the process of speciation and is frequently the first stage rather than the last in the production of a new species" (Gates, 1938). Several cases of single factors or complementary genes producing intersterility are now known and may be briefly cited here. Hollinshead (1930) described the first case, in *Crepis*. Some plants of *C. tectorum* were found to be heterozygous and some homozygous for a single recessive gene which, in crosses with *C. capillaris*, produces seedlings which die at the cotyledon stage. The same lethal effect was produced in crosses of *C. tectorum* with *C. bursifolia* and *C. leontodontoides*. But this factor had no effect in crosses with *C. setosa* and *C. vesicaria*

taraxacifolia. This gene for intersterility was evidently widespread in parts of the distribution of *C. tectorum*, and there are indications of a similar interspecific lethal in certain other species of *Crepis*. Hutchinson (1932) found "crumpled," an abnormal sterile or semi-sterile type arising in *Gossypium Nanking* var. *soudanensis* \times *G. arboreum* var. *sanguinea*. This was due to complementary factors, A present in two strains of *G. Nanking* from the Sudan, and B in seventeen varieties of *G. arboreum*, *G. Nanking*, *G. herbaceum*, *G. obtusifolium* and a strain of *G. Stocksii* from Sind. In some crosses the presence of A and B produced shrivelled or aborted embryos, but modifying factors in Asiatic cottons affected the expression of the crumpled character and improved its viability. A more extreme lethal in *Hutchinsia alpina* produces empty seeds in crosses with *H. brevicaulis* (Melchers, 1939). The gene which prevents fertile crosses with *H. brevicaulis* is present in *H. alpina*, south of Innsbruck, but other races of *H. alpina* were fully fertile in crosses with *H. brevicaulis* from the Dolomites. A race homozygous for the intersterility gene must have arisen through inbreeding.

It is well known that species crosses in *Oenothera* frequently produce a non-viable type which dies in the seedling stage. Several such cases are cited by Gates and Catcheside (1932). Sears (1940) concludes that *Triticum monococcum* contains a dominant gene which is lethal in reciprocal crosses with *Aegilops umbellulata*, and Vavilov recognized such lethals in several crosses between European and Indian soft wheats. Dobzhansky (1941) refers to certain other cases. He regards these single genes as "merely extreme examples of the interdependence of genes composing a balanced genotype" (p. 279), but in any case the evidence shows that single genes may introduce intersterility among forms which are phenotypically indistinguishable. Patterson and Griffen (1944) described a sex-linked gene in *Drosophila texana* and *D. montana* which has no effect in its own species, but the montana egg fertilized by an X-bearing texana sperm produces an inviable female zygote. Here also complementary genes are involved, and a similar condition is present in the cross *D. mulleri* \times *D. aldrichi* δ . Thus single or complementary genes in nearly related forms can produce intersterility and prevent gene interchange.

When the matter of species is considered in its time aspect, from the point of view of palaeontology, it becomes clear that intersterility between contemporary evolving forms is incidental, depending on many chance vicissitudes but more especially on (a) chromosome translocations, deletions, reversals, and other changes, (b) amphiploidy, in which an interspecific hybrid doubles its chromosomes. The former conditions are found equally in plants and animals, whereas the latter occur mainly in plants. The evidence indicates that translocations, leading to linkage groups of genes and to intersterility, are universal in sexual organisms, although their frequency is higher in some plants than in others.

Thus contemporary species of a genus may or may not develop intersterility, or may develop it to very different degrees. On the other hand,

when a species gives rise to one or more other species in geological time, it is obvious that there has been continuous fertility between the mother and daughter species throughout the transition period; except in the case of amphidiploids, where two parent species are involved in producing a stable new species within a short period after contact and crossing. When we contemplate the various other ways in which a condition of sterility can arise within a species, as by the formation of self-sterility genes or apomicty, it becomes clear that intersterility is an incidental phenomenon in evolution. It may arise early or late or not at all in the process of speciation.

There are whole genera or large subgenera of plants in which the species are nearly all interfertile. These probably include the raspberries, *Rubus* (*Idaeobatus*), and *Crataegus* as well as *Cypripedium*² and many others. In *Oenothera* (*Euoenothera*) some 70 species appear to be all interfertile. Interbreeding seldom occurs between them in nature because most of the species of *Oenothera* are self-pollinating in the bud, before insects can reach the flower. Although these species are highly heterozygous, the catenation of the chromosomes, together with the presence of balanced lethals, prevents genetic segregation, so that successive generations breed true. This condition of chromosome catenation is no longer unique but is now known in many plant families, although its genetical effect is not necessarily the same in these genera. Not only in *Gaura* and *Godetia* and other genera of the Onagraceae but in *Hypericum punctatum* (Hypericaceae), *Polemonium* (Polemoniaceae), *Aucuba* (Cornaceae), *Datura* (Solanaceae), *Paonia* (Ranunculaceae), *Humulus* (Cannabinaceae), *Campanula persicifolia* (Campanulaceae), *Pisum* (Leguminosae), *Rosa* (Rosaceae), *Rumex* (Polygonaceae), *Rhoeo discolor* (Commelinaceae) and *Anthoxanthum*, *Avena*, *Briza* and *Zea* (Gramineae) among flowering plants, and also in the Gymnosperms, *Taxus* and *Cephalotaxus*. The conditions which limit, without excluding, interbreeding range from self-pollinating mechanisms, such as that in *Oenothera* or wheat, and cleistogamy, to geographic isolation. They lead to every conceivable degree of partial isolation. It is clear that without isolation in some form speciation cannot take place (except again, in the case of amphidiploids and also in the geological sense where one species gives rise to another as descendant).

CHANGES UNDERLYING SPECIATION

From such facts as those already mentioned we may derive the view that in speciation there are two main streams of change: (a) gene mutations which alter the phenotype, and (b) chromosome changes such as translocations, which generally have no effect on the phenotype but which alter the gene

²In a letter of C. C. Hurst, he wrote in 1896 (R. Hurst, 1949), "with regard to the limits of species, I once fondly thought that fertility and non-fertility would be the true test of species...but my hopes have been sadly dispelled of late, since my experience with orchids." He crossed *Cypripedium barbatum* successfully with 24 distinct species out of some 30 cultivated in gardens. "All are distinct in every way and could not be termed varieties merely." There are two known cases in Orchids in which a hybrid has been built up from crosses made between four genera!

linkages and also introduce intersterility. The existence of these two independent categories of variation results from the fundamental fact that every significant cell has a nucleus, the elements of which can undergo either structural or physiological mutations. Karyotype evolution can thus be largely independent of phenotype evolution, although both take place in the same organisms. It has been pointed out elsewhere (Gates, 1950) that although man in the past has selected only for the phenotype while nature selects for the whole organism at every level of structure, yet this phenotypic selection has resulted in changes of chromosome organization as well. There is no reason to suppose that any necessary relation exists between the frequency of gene mutations and the frequency of chromosome changes such as translocation. Indeed, the evidence indicates that while the former are most readily produced by X-rays and other forms of radiation, the latter often result in nature from excessive temperature effects or humidity in seeds, which cause the chromatin threads to adhere and break. In some plant genera with stable nuclei, such as *Ribes*, *Cistus*, *Carica*, *Philadelphus* and *Streptocarpus*, the evolution of many species has occurred without any change in number or marked visible alteration in the set of chromosomes. Reciprocal translocations and small deletions may nevertheless have taken place. In the genus *Lilium*, in which 45 species are known to have twelve pairs of chromosomes (with a few extra fragments in certain species) the only visible differences are in the number and position of the nucleolar loci (Stewart, 1948). In other genera, such as *Crepis* (Babcock, 1947), the nucleus has been in an unstable condition and many changes in the karyotype, such as alteration in the length of particular chromosome arms, diminution in chromosome number through a form of translocation, and decrease in size of all the chromosomes, have taken place.

These two streams of change, nuclear and phenotypic, occurring in the same lines of descent in a species, will be equally subject to the forces of natural selection. There is evidence that in certain strains of *Drosophila*, individuals which are heterozygous for a certain translocation have a selective advantage over others in the population. This advantage may vary with temperature, thus leading to cyclic changes in the relative frequency of such individuals during different seasons of the year. Similarly it has been shown (Nabours and Stebbins, 1947) in the grouse locust, *Apotettix eurycephalus*, that a gene which is lethal in the homozygous condition produces viability superior to normal in individuals which are heterozygous for this gene. When present in double dose, the individual dies four days before hatching time, whereas a single dose produces individuals which are more vigorous, better feeders and better breeders than the normal. Gustafsson (1946) describes and cites numerous similar cases in both plants and animals, where an individual heterozygous for a lethal gene has superior viability.

Geneticists and taxonomists appear to be agreed that, although type specimens are of course necessary, a species is a dynamic system consisting of a population of living individuals. It is necessary to remember also that time enters as a fourth dimension into the concept of species.

We must then think of a succession of living populations as making up the species, until it finally in the course of time becomes another species or breaks up into two or more specific entities. The consideration of populations leads to other aspects of species on which there is less general agreement. In considering populations of *Vaccinium*, Camp (1942) says, "It is not within the province of the systematist to decide whether a population is worthy of being recognized as a species solely on the basis of its manner of origin; his primary problem is to decide whether it is an agrestial and self-sustaining population morphologically sufficiently distinct from others that it merits this rank." And he recognizes that in this decision some judgment must be used.

Camp also finds it necessary to distinguish between phyletic divergence (basic evolution) and "mere speciation," but such a distinction will probably be obscure in many cases. He believes, no doubt rightly, that many polyploids are outside the stream of basic evolution, but it can scarcely be held that all fundamental evolution is at the $2n$ level. For example, the whole group *Pomoideae*, successful fruit trees, have a derived basic chromosome number $n = 17$, different species being $2n$, $3n$ and $4n$. Similarly, the large and successful genus *Acacia* is based on a derived number, 13, having both diploid and tetraploid species. *Acer* also, a widespread and successful genus of temperate trees, has 13 as basic number, 18 species being diploid, three tetraploid, and in one (*A. rubrum*) individuals have been examined with $6n$ and $8n$ (104) chromosomes. On the other hand, *Houttuynia cordata* with ca. 96 chromosomes is probably at least octoploid and is clearly an isolated survivor, due for future extinction. Similarly *Welwitschia*, a relict *Gymnosperm* which is nearing extinction in the desert of southwest Africa, has chromosome counts of 42 and 84, the latter number being 12-ploid on the basis of 7, the basic number in the related genus *Ephedra*. The optimum degree of ploidy is evidently higher in some genera than in others, and many factors will determine this optimum.

In a further analysis of the nature of species, Camp and Gilly (1943), in an important contribution, recognize thirteen different kinds of populations or species, according to their manner of origin. "It is the genetic structure of a species which makes it a recognizable unit." They go on to say, to "make so broad an interpretation of the species as to include in it all forms which can be shown to be capable of interbreeding and producing fertile offspring, would lead to quite as great an absurdity" as to name as a species every form differing by a single character capable of vegetative or sexual reproduction. Among the kinds of species which they recognize are the homogeneous, a population in which all the members are interfertile and genetically and morphologically homogeneous—a relatively rare condition. Self-explanatory terms are euploidion, allopolyploidion, cleistogameon, heterogameon (heterozygous species, structural hybrids, breeding true, as in *Oenothera*), agameon. The micton consists of hybrids of two or more species, all interfertile. The rheogameon (syngameon or *Rassenkreis*) consists of interbreeding subspecies between which there is more or less

"gene flow." Those who promulgated the International Rules of Botanical Nomenclature in 1935 still adhered to the ancient doctrine that hybrids between species, with but few exceptions, are sterile. It is to be hoped that we have now heard the last of this attempt to equate interfertility or intersterility with species. In general, Camp and Gilly recognize two kinds of species, those which contain accretional elements and those which are of concretionary nature (containing subspecies).

SUBSPECIFIC CATEGORIES

This brings up in acute form the question of the treatment of subspecific categories. Without entering into detail on this subject one may point out that in the case of hybrid swarms any complete nomenclature would have to recognize almost every individual as different from every other. If the population is the unit, then the types within a mixed population should not receive Latin names, but there are exceptions to this rule. In entomology it is suggested (Hatch, 1946) that only populations merit a specific or intra-specific name, (the matter of naming being determined by usefulness and expediency), and that color varieties should receive common but not Latin names. The numerous color patterns in some species of insects renders this problem more acute for entomologists than for botanists, by whom such trivial names as *albida*, *inermis* or *nana*, are frequently used.

That the treatment of hybrid swarms is an important problem is shown, for instance, by the work of Allan (1940) in New Zealand. Cockayne and Allan recorded 491 hybrid groups, and Allan cites many cases of fertile species hybrids and hybrid swarms in the New Zealand flora. He separates them into pauciform and multiform. Later evidence will show that intercrossing, even over long periods, does not necessarily merge the species which intercross. As a widespread condition in nature, hybridization has played an important part in evolution, but the basic material for change has been supplied by gene-mutation and chromosome rearrangements. The frequently reticulate relationships between the characters of Angiosperm families and between smaller units probably involve in part former hybridization.

For units within the plant species the terms subspecies, ecotype, variety and forma have probably been in use most frequently. Camp and Gilly recognize that in practice many species are composed of subspecies. The term ecospecies can be of much value in the analysis of the nature of species, as Clausen, Keck and Hiesey (1939, 1940, 1948) have shown. Continuing and extending the methods of Turesson, by transplanting clones of the same ecotypes of many species to gardens with very different climatic conditions, they consider that ecotypes \rightarrow ecospecies³ \rightarrow coenospecies \rightarrow comparium, represent stages in an evolutionary sequence. They find no genetic barrier between ecotypes but they recognize (1939) that "There actually is a natural reason for the fact that although crossings constantly take place, the species

³Valentine (1949) proposes the following subdivisions: g-ecospecies having the same chromosome number; a-ecospecies with different chromosome numbers; caryotypes, a group forming polyploid components of an ecospecies.

have remained remarkably the same for centuries, as evidenced by herbarium specimens, and even for ages, as shown by geological records." They also agree that "it is impossible to produce definitions that will cover all differences between systematic units, because species are in all stages of evolution." Transitions occur between all the categories mentioned above; but barriers, internal (hereditary) or external (environmental), are the basis of classification. The genetic barriers between coenospecies are regarded as absolute, while a certain amount of gene flow takes place between ecospecies. Such an exchange may give an appearance of parallel variation in related ecospecies. In such genera as *Salix* or *Viola* there may be 20-30 ecospecies in one coenospecies. When experimental evidence is lacking, the terms subspecies, species and species-complex will take the place of the ecological terms. But ecospecies can only be determined with certainty by careful transplant experiments. The analysis of many species from this point of view must await larger facilities than at present exist.

This method is chiefly valuable in showing how precisely different ecotypes of many species, growing for instance at different altitudes, have become adapted to the climatic and edaphic conditions. California is a very favorable region for such experiments. A transect of 200 miles across Central California, from the seashore to the Coast Range, the San Joaquin Valley and the Sierra Nevadas above the tree line, gives an unsurpassed variety of climatic and altitudinal conditions. Clausen, Keck and Hiesey (1948) have applied this method to the genus *Achillea*. The Old World *A. millefolium* L. is hexaploid ($2n = 54$) and widely introduced in America. *A. borealis* Bong., also hexaploid, extends from the Aleutian Islands and Alaska down the coast to Southern California. Three subspecies are recognized, *arenicola* on the coast from Oregon to California, *californica* in the foothills of California, and *gigantea* in the San Joaquin Valley. *A. lanulosa* Nutt. is tetraploid ($2n = 36$) and native from New England to near the Pacific coast, where it meets *A. borealis*. Where these species meet they can only be distinguished by their chromosome number, but it is considered best to retain them as species because of their geographical distribution. The two native American forms are separated by trivial characters of leaf cut and vesture. The distribution was determined by 108 chromosome counts made by Lawrence (1947). A single location in California was octoploid.

The authors collected *Achillea* seeds from 80 localities, grew 60 seedlings from each, and selected 30 of these showing the entire range of variation, for clonal division. The clones were transplanted to gardens at Stanford near sea-level, Mather in the Sierras at 1400 m., and Timberline on the eastern Sierra slopes at 3000 m. In this way a surprising degree of genetic diversity was disclosed. "Almost every type of climate (in California) except the true desert has its race of *Achillea*," each being particularly well fitted to its native climate both in form and in developmental processes. Climatic, edaphic and biotic races are involved, the climate largely determining the other two. Each ecological race approaches an

equilibrium with the environment, variations in the organism being balanced against natural selection acting through environmental factors. The variation in size alone is from lush valley forms to dwarf alpine, but neither will survive in the habitat of the other. The dynamic equilibrium of each local ecological race is nevertheless somewhat flexible, through the morphological and physiological diversity of its component individuals, scarcely two of which belong to the same biotype. There are at least eleven diverse climatic races along this 200 mile transect, each having a vast amount of genetic variability. In *Antirrhinum majus* Melchers (1939) has described a series of ecological mutants, *heroína* (tall), *eramosa* (unbranched) *nana* (dwarf), *alpina* (branched from the base), *matura* (with early flowers) and *densiflora* (with many grouped flowers). These, as well as Turesson's earlier work, show how ecotypes can have arisen through mutation.

In a similar study of the Californian *Potentilla glandulosa* Lindl., Clausen (1949) has used not only transplantation but crossing experiments. Many diverse ecotypes are found belonging to 11 or 12 distinct subspecies and about 8 climatic races in a transect of Central California. Although morphologically distinct, all are completely interfertile, having $n=7$ chromosomes. In the Coast Range is subsp. *typica*; in the foothills of the Sierras *P. reflexa* Greene is reduced to a subspecies; *P. Hanseni* Greene occurs in meadows at higher altitudes; and *P. nevadensis* S. Wats. is another subspecies at alpine levels.

In transplants to the same three stations, *typica* and *reflexa* die at Timberline, while *Hansenii* survives at all three but develops best at Mather. Crosses between these three subspecies give an F_1 which is vigorous and fertile. In the F_2 , for example, of *typica* \times *nevadensis*, every recombination occurs. These were cloned and grown for 7 to 9 years at the three stations, and 20 F_3 progenies were produced. From a detailed analysis of the F_2 , 60-100 genes were estimated to be involved. There were several genes (usually 2-4) for each character, and the physiological as well as the morphological characters were thus graded. The petal color ranges from white to yellow, and five pairs of genes for color were estimated to be present in crosses between the foothill and the alpine form. The F_1 had a wider tolerance than the parents, and could survive at all stations. All kinds of growth patterns were produced through gene exchange, including "super-alpines" which could thrive best in alpine conditions. The physiological and morphological characters were inherited independently so that some physiological alpine have the morphological traits of the foothill forms. Some recombinations were weak at all stations while some were successful in all, showing wider tolerance than any wild form. The ecotypes are thus as diverse as well recognized subspecies, but they are determined by many genes. Crosses between them, which are rare in nature, release new evolutionary possibilities.

I have referred to this work in detail because its carefully planned experiments, carried out on a large scale, throw much light on the nature of specific and subspecific variability. The three main forms, although mor-

phologically distinct, are physiologically adapted to different habitats, and although fully interfertile rarely cross in nature. This is a typical borderline case where opinions will differ as to whether the three forms should be ranked as species or subspecies. The lumpers and splitters will differ on this point. Turrill (1936) cites a clear case where two species which readily intercross are prevented from merging by their different ecological adaptations. *Silene alpina* grows on screes in the French Alps, *S. Cucubalus* in meadows and woodland-edge. Seeds of *S. alpina* collected 50-100 m. above the meadows yielded 12 per cent. of hybrids, and in gardens they cross reciprocally. But they are kept separate by the physiographic, edaphic and biotic differences in their habitats.

The Case of Crepis

In his world monograph of the genus *Crepis* (already cited), based on thirty years cytogenetic, geographic and taxonomic work with the genus, Babcock (1947) disagrees with the point of view of Clausen, Keck and Hiesey that classification as species or subspecies should be based primarily on intersterility. He points out that the consensus of plant systematists is against it. He cites the case of *C. foetida*, *C. eritreënsis* and *C. Thomsonii*, which are highly interfertile but geographically isolated. He further finds it impossible to apply the concepts of ecotype, ecospecies, coenospecies and comparium in the genus *Crepis*. With a world background, the comparium (species which intercross to produce sterile hybrids) would contain species from many different sections of the genus.

In this extensive monograph the subgenera of Bentham and Hooker have been discarded as not conforming with the natural relationships of the species. Instead, the 196 species recognized (including 33 described as new) are divided into 27 sections. The genus is monophyletic except two sections which originated through hybridization with the ancestors of different genera. Evolution in the genus has been from species which are "robust, rhizomatous, mesophytic perennials with large simple fruits and short-lived seeds," to "small, delicate desert annuals, with their extreme precocity, ephemeral fibrous taproot, tiny fruits with a filamentous beak, and seeds of longer viability." In the genus, 143 species are perennial, 40 annual, the latter certainly derived, as is the taproot from the rhizome. Nodding versus erect heads, and the presence or absence of paleae on the receptacle, are each determined by a single gene.

The genus is probably derived from *Dubyaea* (with $n = 8$ chromosomes). In *Crepis* there has been a diminution of the chromosomes by steps, from $n = 6$ in primitive species to 5, 4 and finally 3 in the most advanced, one section alone being transitional, with $n = 7$. "Each step in decrease occurred independently of other steps and on several different occasions" (Babcock, 1947, p. 146), that is, these changes have been repeated and parallel. The reduction from 5 to 4 occurred at least four times, 58 species now having $n = 4$. This reduction in number has been accomplished by non-homologous reciprocal translocations involving the loss of a kinomere and

a chromosome fragment. Other translocations account for the increasing asymmetry in the length of chromosome arms. There is in addition a progressive decrease in size of all the chromosomes, which is correlated, at least in part, with diminution in size of the plant.

These lines of evolution have occurred in the Old World. The North American group (Section 15) have as basic number $n = 11$ chromosomes. They must have arisen as amphidiploids from Asiatic species having 7 and 4 or 6 and 5 chromosomes respectively. This group, confined mainly to Western North America, has also developed higher polyploidy (up to $2n = 88$) as well as apomixis, whereas very few of the Old World species are polyploid and none are known to be apomictic. Change in chromosome number in the diminishing series (e.g., $5 \rightarrow 4$) is not necessarily accompanied by much phenotypic change. However, it provides the basis for differentiation of species by gene mutations, which has been the chief agent of change in phenotype. The fact that 59 per cent. of the species of *Crepis* overlap with related species in their distribution shows how important nuclear changes have been in producing isolation. At the same time 133 of the 196 species are listed as endemic.

The net result of all the experimental work in cytogenetics and ecology is thus to show that while intersterility is an important criterion in determining taxonomic units, its application as a universal criterion would result in chaos, because intersterility is itself so sporadic in its development.

There is much diversity of opinion regarding the relative value of the concepts "subspecies" and "variety." Both terms, but especially the variety, have undergone changes of usage. R. T. Clausen (1941) strongly advocates following the custom of zoologists and using the subspecies simply as a trinomial attached to the specific name. This method appears to be not entirely in harmony with the needs of taxonomic botany. It would relegate the variety to its original more trivial use, as in horticulture and agriculture. There seems a place for both terms, *subspecies* being applied to forms which show more complex differences in correlation with the factors of geographical, ecological and physiological isolation, *variety* to differences of less significance.

SPECIES CRITERIA

To return to the question of the delimitation of species, most naturalists who have had wide experience in the taxonomy of any group of organisms appear to have reached finally the view that there is no criterion for a good taxonomist except that he have wide knowledge of his particular group, and good judgment. Thus Darwin wrote that "the opinion of naturalists having sound judgment and wide experience seems the only guide to follow," and Tate Regan (quoted in Gates, 1948, p. 378), an authority on fishes, came to a similar conclusion. Hitchcock (1925), a leader in the taxonomy of grasses, wrote, "The term (species) is difficult to define with precision because a species is not a definite entity but a taxonomic concept. Where a line shall be drawn is often a matter of convenience in classification." In cases where

two populations are connected by scattered intermediate individuals, "Botanists will differ as to whether we have here two species with intermediate individuals, one species with two varieties, or subspecies, or one variable species.... the actual delimitation of a species is a matter of judgment and experience." Babcock (1947, p. 34) writes in the same vein, "decisions concerning just how much difference between populations shall be sufficient for the recognition of a new species had best be left to the judgment of biosystematists who specialize in the various fields of organic life."

Harland (1936), after referring to the definitions of Ostenfeld, Vavilov, Hitchcock, Baker and Calman, concludes with the general consensus of opinion that it is not possible to define species in all cases, and that any attempt at definition should be in as general terms as possible. From his experience especially with the genus *Gossypium* he concludes that if two species or even genera can be crossed and produce fertile offspring there is always evidence that "either identical or at least homologous genes lie in homologous loci in the species involved." In six species of *Gossypium* he finds allelic relationships in all the genes examined and calls them "species alleles," but he regards the various modifiers as species differentials. His species alleles are thus not the same as the interspecific genes of Lamprecht (1948). The species differ not only in their main gene alleles but also in their complexes of modifiers, which are of primary importance from the taxonomic point of view. Gene substitution has then been the main mechanism of species differentiation in *Gossypium* through isolation.

There will doubtless always be lumpers and splitters according as the individual is more impressed by the resemblances or the differences between organisms involved. This is partly a matter of temperament, and the two activities involved are largely complementary. The splitter recognizes numerous smaller units, which the lumper combines so as to show their relationships. The activities of the splitter are necessary in connection with the genetic, cytological and ecological analysis of the subunits composing many species. The synthesis of the lumper is equally useful in reaching an understanding of the evolutionary history and relationships of any group of organisms.

ISOLATING MECHANISMS

All geneticists and most taxonomists will probably agree that the role of isolating mechanisms is fundamental in the differentiation of contemporary species. Stebbins (1942) has ably discussed this subject and expressed views which are, in the main, in agreement with those developed in the present paper. He recognizes that internal isolating mechanisms are genetically independent of morphological (phenotypic) changes, and that an understanding of them depends on knowledge of the many types of external and internal isolating mechanism which exist. He points out that geographic isolation does not necessarily lead to phenotypic divergence, and cites from the work of Fernald the striking cases of *Symphoricarpus foetidus*, *Cypripedium arietinum* and three species of *Polygonum*, which are identical

in the eastern United States and in eastern Asia although they have been thus widely separated since the mid Tertiary. Equally striking cases are cited in *Catalpa* and *Platanus*. *P. orientalis* of Asia Minor crosses with *P. occidentalis* of the eastern United States to produce the London plane, frequently called *P. acerifolia*, which is fully fertile and exceptionally hardy, the chromosomes of the hybrid pairing normally in meiosis (Sax, 1933), its seedlings segregating in F_2 . This genus goes back to the Cretaceous and was formerly distributed over nearly the whole northern hemisphere, many fossil species being recorded from Cretaceous and Tertiary deposits. These two surviving species must have been geographically separated for millions of years, yet the number of chromosomes ($n = 21$) in both species indicates either hexaploidy⁴ or an origin through amphidiploidy, and the chromosomes appear to be of uniform size except for one possible smaller pair. The specific differences are imperfectly described but they pertain to every part of the tree. The number of gene pairs involved is roughly estimated as follows: habit of the tree (2), bark (1), hardiness (1), leaves (2), stipules (4), petioles (3), inflorescence (1), calyx (1), petals (1), making a total of at least 16 genic differences. It would seem that in this primitive Angiosperm genus at any rate a large amount of differentiation has taken place without any loss of interfertility. The florets are of extremely simple structure although their aggregation into a head is a specialization, but this has happened independently, e.g., in the genus *Parkia* of the Leguminosae as well as in the Compositae. Such cases lead one to doubt whether there is any limit to the number of gene pairs for which an organism may be heterozygous without impairing its fertility.

As regards *Paeonia*, which he has studied extensively, Stebbins (1942) believes the evidence "is definitely against the assumption that interspecific sterility results chiefly from gross structural changes" in the chromosomes. However, *Paeonia*, like *Oenothera*, has indulged in the form of chromosome interchange which leads to complete catenation and is not accompanied by intersterility. He thinks that intersterility in general has been built up by many small changes the nature of which is unknown. He finds that in various cases "The visible characteristics which differentiate the species and the sterility barriers which partly isolate them segregate quite independently of each other." In rice, on the contrary, he points out that some races which are very different morphologically are interfertile and some races which closely resemble each other are intersterile. This is exactly to be expected if, as I have suggested, chromosome changes leading to intersterility, and gene mutations in the phenotype, are independent in their origin and effect. Yet, as Stebbins states (p. 230) that "there is no direct correlation between the genetic isolating mechanisms which produce

⁴The three related families, Magnoliaceae, Trochodendraceae and Cercidiphyllaceae have $n = 19$, one species being tetraploid on this basis. This number 19 is probably amphidiploid in origin, indicating that, although the flower type is primitive among Angiosperms, the chromosome number is "advanced." This and other evidence increases the pressure to regard the Angiosperms as having a polyphyletic origin.

hybrid sterility and the genetic changes which make species visibly different," it appears that very little difference remains between his views and mine. Later, (1945), he appears in places to have succumbed to the view we are combatting, that intersterility or isolation factors can be fully equated with species. However, we assume that we would not agree with Dobzhansky in describing any forms which do not cross as new species, regardless of the absence of adequate phenotypic difference.

Further evidence that a great amount of differentiation between species can occur by gene mutations without loss in fertility is found in the work of Baur (1932) on *Antirrhinum*. He collected *Antirrhinums* all over Spain and the Western Mediterranean region. He found (1) a few local sharply isolated colonies representing our well-characterized species, (2) a series of nine large *Formenkreisen*, each composed of innumerable local races, generally of one type but connected by series of intermediates. The plants in some of these colonies were very heterozygous. *A. majus* grew wild in Southern and Middle Italy, Sicily, Algeria, Morocco, and the Iberian peninsula. Three genes of *A. hispanicum* were identical with those of *A. majus*. Baur concluded that over a hundred genes segregated in the fully fertile F_2 cross of these two species. Kühl (1938) afterwards published a further analysis showing that eight local wild species were all homozygous and hence stable, yet the interspecific crosses were fully fertile. Eleven genes for flower characters were recognized.

SPECIES HYBRIDIZATION

On the other hand, the analysis of the genus *Zauschneria* on the coast of California by Clausen, Keck and Hiesey (1940) shows that *Z. septentrionalis* in the north and *Z. cana* further south can be crossed readily, providing a fertile F_1 , but the F_2 consists only of weak individuals. In both species $n = 15$. In nature, were these species sympatric, backcrosses would no doubt produce a hybrid swarm mainly of weak individuals, but particular combinations might be strong enough even to replace a parent species. Keck's description of the northern form as a new species is no doubt justified, as it has matted herbaceous (not woody) stems, broader (not filiform), subentire and densely canescent leaves.

The English primrose (*Primula vulgaris*) and oxlip (*P. elatior*) occur in the same woods in East Anglia and intercross, but each wood is isolated. Ecological preferences keep the species mainly in different parts of the wood (Valentine, 1949a). Reciprocal crosses were made, which gave a low yield of viable seeds. The vigor of the F_1 is variable, but when it is selfed or backcrossed a better yield of seeds is obtained. In this case ecological and genetic barriers prevent fusion of the species, the mixed populations appearing to be in equilibrium.

The genus *Balsamorhiza*, containing about a dozen species in the Western States, shows various conditions of intercrossing (Ownbey and Weber, 1943). *B. sagittata* and *B. Careyana* have slightly different habitat preferences, but at a point of contact intermediates (hybrids ?) were found. *B. Careyana* and

B. deltoidea on the contrary merge completely (with the formation of intermediates) where they meet. The extremes of the series are species, morphologically and ecologically, but biologically they are "parts of the same unit," and all attempts to separate them are futile. Although hybridization has obscured the discontinuity which perhaps existed originally, yet it has not submerged either species. Some of the other species do not meet and therefore do not intercross, yet members of two very distinct subgenera hybridize when in the same neighborhood, producing hybrid swarms with all the character recombinations. The authors reach the conclusion that "hybridization between widely separated entities may not indicate the desirability of a complete revision of time-tested taxonomic concepts so much as a need for better understanding of the nature and development of genetic barriers"... "the axiomatic application of hybrid sterility or inviability to the problem of species delimitation may be as unsatisfactory as the axiomatic application of the criterion of morphological difference." Evolutionary divergence between populations is a measure of the effectiveness of the barrier between them, not of its nature. Distinctions between geographic and genetic barriers may have no significance in evolution or in systematics.

We may next consider the tree genus *Catalpa*. *C. ovata* Don is native to China, *C. bignonioides* Walt. to North America. The reciprocal hybrids, known as $\times C. hybrida$, are fertile, the F_1 showing hybrid vigor and the F_2 segregating. All have $2n = 40$ chromosomes. In the hybrids, as in the parents, the pollen meiosis is regular (Smith, 1941). These two species have been separated geographically for geological ages, yet they differ only by gene mutations and no taxonomist would unite them because of their interfertility. A fossil *Catalpa* occurs in the Oligocene of the Isle of Wight.

The careful study of hybridization in nature thus throws so much light on the question of species relationships that a few more specific cases may be cited from the many in the literature. The paleontological aspects of species enters into a case in the oaks, described by Chaney (1949). *Quercus wizlizenii* A. DeCand., the interior live oak of California, hybridizes with *Q. kelloggii* Newberry, the California black oak, producing *Q. morebus* Kellogg, "a species restricted in distribution and numbers." The Tertiary equivalents of the parent species occur in the Miocene—Pliocene flora of Central California, and a third species, *Q. remingtoni* Condit, is probably the hybrid between them. The evidence indicates that hybridization was taking place on the western slopes of the Sierras several million years ago as it is now between the modern descendant species. Yet they have not merged, and *Q. remingtoni* is not a direct ancestor of *Q. morebus* but a Tertiary hybrid black oak.

Another instance where the "fourth dimension" (time) enters in is described by Mason (1949) in *Pinus*. There is evidence that *P. remorata* and *P. muricata* were distinct species in the Pleistocene of California. *P. remorata*, now confined to Santa Cruz island, is near extinction. *P. muricata* on the mainland occurs discontinuously in colonies along the opposite coast. The prevailing east winds carried the pollen of *P. remorata*, which

thus introduced genes of this species into the southern range of *P. muricata*. The latter species has probably reached the island since, where the two species now form hybrid swarms. In this way *P. remorata*, which was formerly a "good" species, has been practically eliminated on the island.

Another case in which natural interspecific crossing has affected the phylogenetic development of both species is that of guayule (*Parthenium argentatum*) and mariola (*P. incanum*). These species cover much the same range in northern Mexico and western Texas. They mingle freely and there has been much introgression of genes (Anderson). From wild seeds collected, Rollins (1944) grew over 205,000 plants of guayule and nearly 1,000 of mariola. The natural hybrids numbered 162 in guayule, or less than 0.1 per cent., and in mariola only one plant. The trichomes of the two species are radically different, and this was used as an index of hybridity. The two species also differ in foliage and growth habit, and mariola has smaller heads and florets, the ray florets less deeply divided. The amount of bad pollen in each species varies from 5 per cent. to over 50 per cent. Both species show chromosome numbers which range in guayule from $2n = 36$, to 54, 58, 72, 74 and 108-112, and in mariola from 36 to 54, 72, 81, 90. Polyploidy and aneuploidy are both involved in this process of introgression. Some hybrids have very good pollen, others show the usual meiotic irregularities. From all the evidence, it is highly improbable that these two species evolved in separate areas and have since come together. Rather, evolution appears to have taken place, including polyploidy and finally apomicty, under conditions of continuous introgression.

A case with very interesting features is in the genus *Solidago* (Goodwin, 1937). *S. rugosa* Mill. and *S. sempervirens* L. are two very distinct species, and $\times S. asperula$ Desf. has long been recognized as the hybrid between them. *S. rugosa* inhabits open meadows and *S. sempervirens* salt-marshes, having the characteristic fleshiness of halotypes such as *Aster tripolium*. Plants were collected and inbred in the greenhouse for two generations, both species being constant. Reciprocal crosses were made, both species having $n = 9$ chromosomes. Meiosis was normal in F_1 , F_2 and backcrosses, and there was no aberrant pollen sterility. The differences between these species are not only in habitat, but also in the stolons, leaves, pubescence, inflorescence and various characters of the flowers and heads. Goodwin found six inherited differences in the leaves alone, and one may estimate at least 28 character differences. As these frequently depend on multiple quantitative genes, the number of interspecific genes must be much greater. The presence of ecological adaptation as well as marked morphological differences between two species which are nevertheless fully fertile shows how futile it would be to use interfertility as a better criterion of species than morphological difference.

A pair of species in *Lactuca* (Whitaker, 1944) are of interest because "These two very distinct species are for the most part dependent upon simple gene differences." *L. graminifolia* and *L. canadensis* were crossed, the F_1 and later generations being as fertile as either parent species, their

meiosis normal. The former species occurs from South Carolina to Florida and Texas, the latter from Nova Scotia to Saskatchewan and south to Arkansas, Louisiana and Georgia. Natural hybrids are found in South Carolina where their very different ranges overlap. Both species were uniform in cultures and reciprocal crosses were made. The F_1 is more or less intermediate, but the annual habit of *graminifolia* is dominant to the biennial habit of *L. canadensis*. The grey pollen (versus orange) and purple blue (versus orange yellow) ligules of *L. graminifolia* were dominant, the pollen color segregating very sharply as a sporophytic character. The lobed, pinnatifid (versus entire, lanceolate) leaves of *L. canadensis*, were dominant, but modifying factors determined the nature of the lobing. *L. canadensis* is much taller but with shorter involucral bracts, the F_1 being intermediate in these respects. In other characters the differences were more complex. This is a good example of a pair of species with four or five very marked differences and probably a large number of minor ones. There is very little hybridization, because *L. graminifolia* flowers earlier than *L. canadensis*. The hybrids are also regarded as unsuccessful because of "their inability to encounter an ecological niche to which they are well adapted." These two species have $n = 17$ chromosomes and they probably diverged from a common amphidiploid ancestor produced by a cross between species having 9 and 8 chromosomes respectively. It would be ridiculous to combine these species or reduce one to a subspecies because their hybrids are fertile.

By way of comparison, a couple of recent cases from animals may be cited. The butterflies, *Basilarchia arthemis* and *B. astyanax* intercross on the line where they meet, in a zone from New England to Pennsylvania, northern New York, central Michigan, Wisconsin, and Minnesota (Hovanitz, 1949). The hybrids produced go under the name *B. proserpina*. This state of affairs was known in 1879 and is the same today, but the species do not merge. Hovanitz cites similar conditions in *Colias philodice* and *C. eurythema*, which overlap in much of their area and intercross, so that some 10 per cent. of the population may be intermediate hybrids. The F_2 swarm are multifactorial and backcrosses are fertile, but the species are not obliterated. This is partly because they have different food plants and there is a high mortality of the hybrids on the wrong food.

According to Shrode and Lush (1947), zebu cattle (*Bos indicus*) are no more sterile in crosses with European cattle (*Bos taurus*) than are the corresponding intraspecific crosses. "They differ widely in many kinds of characteristics, including things as different as horns, skull shape, dewlap, voice, size of digestive tract, resistance to some tropical diseases, and mental traits." They also differ in the presence of the zebu shoulder hump, not to mention the marked differences in coat color. Yet the authors suggest that because these two species, which must have evolved and become domesticated in widely separate areas (one tropical and the other temperate) are interfertile, "probably this should not be called a species cross." I submit that to merge them when morphology and physiology speak so loudly to the contrary is a misuse of the fact of interfertility.

The recognition and naming of plant and animal kinds must have existed from the time man began to speak. The categories of taxonomy have become progressively more exact since that time. Modern taxonomy fulfils this function of classification as well as the intricacies of evolutionary processes will permit. With the recognition of evolution, taxonomy acquired a second function, the building up of phylogenetic lines on the basis of evolutionary theory. Neither of these processes can ever be complete, but every taxonomist recognizes that there are clearly defined species and species which the taxonomist must define by "drawing a line somewhere." The latter cases must always lead to differences of opinion, but it is clear from the modern genetical and cytological work that mere divergence does not necessarily produce intersterility. It is equally clear that intersterility can arise in the absence of phenotypic difference. Since the whole function of taxonomy is based on the recognition and discrimination of phenotypes, the taxonomist must always have the last word in determining what is a species. In taxonomic work there is no rule-of-thumb criterion, such as sterility, which will take the place of wide experience and careful judgment.

LITERATURE CITED

- Allan, H. H., 1940, Natural hybridization in relation to taxonomy. In *The New Systematics* (Ed. J. Huxley). pp. 515-528.
- Babcock, E. B., 1947, The genus *Crepis*. Part I. The taxonomy, phylogeny, distribution, and evolution of *Crepis*. Univ. of Calif. Publ. Botany 21: 1-197.
- Part II. Systematic treatment. *Ibid.* 22: 199-1030.
- Baur, E., 1932, Artumgrenzung und Artbildung in der Gattung *Antirrhinum*, Sektion *Antirrhinastrum*. Z. i. A. V. 63: 256-302.
- Burla, H., A. B. DaCunha, A. R. Cordeiro, T. Dobzhansky, C. Malogolowkin, and C. Pavan, 1949, The Willistoni group of sibling species of *Drosophila*. *Evolution* 3: 300-314.
- Camp, W. H., 1942, On the structure of populations in the genus *Vaccinium*. *Brittonia* 4: 189-204.
- Camp, W. H. and C. L. Gilly, 1943, The origin and structure of species. *Brittonia* 4: 323-385.
- Chaney, R. W., 1949, Evolutionary trends in the Angiosperms. pp. 190-201. In *Genetics, Paleontology and Evolution*. Princeton University Press.
- Clausen, J., 1949, Genetics of climatic races of *Potentilla glandulosa*. pp. 162-172 (*Hereditas Suppl. Vol.*) Proc. 8th Internat. Congr. Genetics.
- Clausen, J., D. D. Keck, and W. M. Hiesey, 1939, The concept of species based on experiment. *Amer. J. Bot.* 26: 103-106.
- 1940, Effect of varied environments of Western North American plants. pp. 452. Carnegie Inst. Publ. No. 520.
- 1948, Environmental responses of climatic races of *Achillea*. pp. 129. Carnegie Inst. Publ. No. 581.
- Clausen, R. T., 1941, On the use of the terms "subspecies" and "variety." *Rhodora* 43: 157-167.
- Dobzhansky, T., 1941, *Genetics and the Origin of Species*. pp. 446. Columbia University Press.
- 1946, Complete reproductive isolation between two morphologically similar species of *Drosophila*. *Ecology* 27: 205-211.
- Gates, R. R., 1936, Genetical and taxonomic investigations in the genus *Oenothera*. *Phil. Trans. Roy. Soc.* 226B: 239-355.

- 1938, The species concept in the light of cytology and genetics. Amer. Nat. 72: 340-349.
- 1948, Human Ancestry from a Genetical point of view. pp. 422. Pls. 27. Harvard University Press.
- 1950, Wild cabbages and the effects of cultivation. J. Genet. (in press).
- Gates, R. R., and D. G. Catcheside, 1932, Gamolysis of various new *Oenotheras*. J. Genet. 26: 143-178.
- Goodwin, R. H., 1937, The cytogenetics of two species of *Solidago* and its bearing on their polymorphy in nature. Amer. J. Bot. 24: 425-432.
- Gustafsson, A., 1946, The effect of heterozygosity on variability and vigour. Hereditas 32: 263-286.
- Harland, S. C., 1936, The genetical conception of the species. Biol. Revs. 11: 83-112.
- Hatch, M. H., 1946, Nameability in taxonomy. Entomol. News 57: 141-143.
- Hitchcock, A. S., 1925, Methods of descriptive systematic botany. pp. 216. New York.
- Hollingshead, L., 1930, A lethal factor in *Crepis* effective only in an interspecific hybrid. Genetics 15: 114-140.
- Hovanitz, W., 1949, Increased variability in populations following natural hybridization. pp. 339-355. In Genetics, Paleontology and Evolution. Princeton University Press.
- Hurst, Rona, 1949, The R.H.S. and the birth of genetics. J. Roy. Hort. Soc. 74: 377-390.
- Hutchinson, J. B., 1932, "Crumpled": a new dominant in Asiatic cottons produced by complementary factors. J. Genet. 25: 281-291.
- Huxley, J., 1940, The New Systematics. pp. 583. Oxford University Press.
- Kühl, O., 1938, Genanalyse bei *Antirrhinum*-Artbastarden. Z.i.A.V. 74: 125-160.
- Lamprecht, H., 1948, Zur Lösung des Artproblems. Agri. Hort. Genetica. 6: 87-141.
- Lawrence, W. E., 1947, Chromosome numbers in *Achillea* in relation to geographic distribution. Amer. J. Bot. 34: 538-545.
- Mason, H. L., 1949, Evidence for the submergence of *Pinus remorata*. pp. 356-362. In Genetics, Paleontology and Evolution. Princeton University Press.
- Melchers, G., 1939, Genetik und Evolution. Z.i.A.V. 76: 229-259.
- Muller, H. J., 1942, Isolating mechanisms, evolution and temperature. Biol. Symposia 6: 71-125.
- Nabours, R. K. and F. M. Stebbins, 1947, More or less potency of genes. Trans. Kansas Acad. Sci. 50: 327-330.
- Ownbey, M. and W. A. Weber, 1943, Natural hybridization in the genus *Balsamorhiza*. Amer. J. Bot. 30: 179-187.
- Patterson, J. T. and A. B. Griffen, 1944, A genetic mechanism underlying species in isolation. Univ. of Texas Publ. No. 4445: 212-223.
- Rollins, R. C., 1944, Evidence for natural hybridity between guayule (*Parthenium argentatum*) and mariola (*P. incanum*). Amer. J. Bot. 31: 93-99.
- Sax, K., 1933, Species hybrids in *Platanus* and *Campsis*. J. Arn. Arb. 14: 274-278.
- Sears, E. R., 1940, Monofactorially conditioned inviability of an intergeneric hybrid in the Triticinae. Genetics 25: 134.
- Shrode, R. R., and J. L. Lush, 1947, The genetics of cattle. Adv. in Genetics. 1: 209-261.
- Smith, E. C., 1941, Chromosome behavior in *Catalpa hybrida* Spaeth. J. Arn. Arb. 22: 219-221.
- Stebbins, G. L., Jr., 1942, The role of isolation in the differentiation of plant species. Biol. Symposia 6: 217-233.
- 1945, The cytological analysis of species hybrids. II. Bot. Rev. 11: 463-486.

- Stewart, R. N., 1948, The morphology of somatic chromosomes in *Lilium*. *Amer. J. Bot.* 34: 9-26.
- Turrill, W. B., 1936, The expansion of taxonomy with special reference to Spermatophyta. *Biol. Revs.* 13: 342-373.
- Valentine, D. H., 1949a, Interspecific hybridization in *Primula*. *Heredity* 3: 128.
- 1949b, The units of experimental taxonomy. *Acta Biotheoretica* 9: 75-88.
- Whitaker, T. W., 1944, The inheritance of certain characters in a cross between two American species of *Lactuca*. *Bull. Torr. Bot. Club* 71: 347-355.

GENETIC TUMORS IN NICOTIANA

AUGUST E. KEHR

Louisiana State University, Baton Rouge, Louisiana

INTRODUCTION

Most plant tumors originate directly as the result of a chain of reactions started by some environmental tumor-inciting agent. Tumors in this category include those stimulated by bacteria, viruses, insects, nematodes, selected chemicals including plant growth regulators, and other factors of external origin which are not generally passed on in the progeny of the plant. Such tumors are never spontaneously initiated from within the plant but are always dependent on factors external to the plant for their inception.

Genetic tumors, on the other hand, arise spontaneously, are initiated solely by factors within the plant cells, and always occur in entire populations of plants of a particular genotype as, for example, in certain interspecific hybrids of *Nicotiana*. In many respects these plant tumors are the counterpart to cancer in animals, notably, both occur spontaneously and the incidence of both is highest among organisms that have passed maturity. Evidence is presented in this study which is indicative that these tumors are controlled by genes affecting the growth regulatory mechanism and appear when the system is thrown out of balance in plants of certain genetic constitution.

EXPERIMENTAL RESULTS

In taking field notes on a total of more than sixty different interspecific hybrids of *Nicotiana* it was observed that certain ones consistently developed tumors. Some of these tumorous growths were strikingly large, up to 50 mm. in diameter, and occurred on almost all parts of the plant (fig. 2). Wounding did not encourage tumor formation if the terminal growing point was not injured. When the terminal buds were removed from actively growing plants, tumors appeared within ten days, while plants that were wounded by cutting halfway through the stem continued growing normally.

The tumors in most instances were not fatal to the plant and usually appeared after flowering was well advanced and terminal meristematic growth had become less active. No tumors were found as long as the plants were in an actively growing condition, an observation that was also made by other workers (Whitaker, 1935, Kostoff, 1943). The stems adjacent to the tumors never produced additional flowers, but commonly new shoots started from the basal parts of the plant and these eventually bore flowers (fig. 4). In some hybrids the tumors appeared at an early age when the plant had only one or two true leaves (figs. 1 and 9). At this stage the plants were not actively elongating.

TABLE 1

INTERSPECIFIC HYBRIDS OF NICOTIANA WHICH WERE PREVIOUSLY REPORTED
TO FORM GENETICALLY CONTROLLED TUMORS

Female parent	Somatic chromosome number	Male parent	Somatic chromosome number	Worker
<i>N. glauca</i>	24	<i>N. langsdorffii</i>	18	Kostoff 1943
<i>N. glauca</i>	24	<i>N. longiflora</i>	20	Kostoff 1943
<i>N. glauca</i>	24	<i>N. plumbaginifolia</i>	20	Kostoff 1943
<i>N. langsdorffii</i>	18	<i>N. glauca</i>	24	Kostoff 1943
<i>N. miersii</i>	24	<i>N. langsdorffii</i>	18	Kostoff 1943
<i>N. paniculata</i>	24	<i>N. langsdorffii</i>	18	Kostoff 1943
<i>N. paniculata</i>	24	<i>N. sanderae</i>	18	Kostoff 1943
<i>N. quadrivalvis</i>	48	<i>N. sanderae</i>	18	Whitaker 1934
<i>N. rustica</i>	48	<i>N. cavanillesii</i>	24	Kostoff 1943
<i>N. rustica</i>	48	<i>N. sanderae</i>	18	Kostoff 1943
<i>N. rustica</i>	48	<i>N. alata</i>	18	Kostoff 1943
<i>N. rustica</i>	48	<i>N. langsdorffii</i>	18	Kostoff 1943
<i>N. rustica</i> var. <i>humilis</i>	48	<i>N. tabacum</i> var. <i>sanguinea</i>	48	Kostoff 1930
<i>N. suaveolens</i>	32	<i>N. langsdorffii</i>	18	Kostoff 1930
<i>N. tabacum</i> var. <i>wigandioides</i>	48	<i>N. sanderae</i>	18	Kostoff 1930
<i>N. tabacum</i> var. <i>sanguinea</i>	48	<i>N. alata</i>	18	Kostoff 1930

In a few hybrids, tumors were found only on the roots of a part of the population. These tumors were identical in appearance to those found on the roots of plants with above-ground tumors and also resembled those caused by crown gall bacteria (fig. 11). However, all attempts to isolate causative pathogens from tissue plantings taken from various portions of these root tumors and grown on nutrient agar were unsuccessful. In one case a plant with root tumors later developed characteristic stem tumors when it was transplanted from the field to the greenhouse. It would appear, therefore, that the root tumors are early manifestations of the physiologic condition that later produces typical stem tumors, but that the above ground parts of the plant do not develop typical stem tumors as long as the plant is actively growing or has not reached the point of physiological maturity required for

FIGURE 1. Tumors on a month-old seedling of the F_1 interspecific hybrid, *N. glauca* \times *N. langsdorffii*, after colchicine treatment. Such seedlings later recover and grow normally until the late flowering period when they again form tumors. ($\times 3$)

FIGURE 2. Close view of tumors on the F_1 hybrid of *N. glauca* \times *N. langsdorffii*, showing nature of tumorous growths. ($\times \frac{1}{2}$)

FIGURE 3. Plant with triple genome combination, *N. debneyi-glauca* \times *N. langsdorffii*, with characteristic tumorous growths. This plant had only *N. debneyi* cytoplasm, but readily formed tumors soon after blooming. ($\times \frac{1}{10}$)

FIGURE 4. Amphidiploid, *N. glauca-langsdorffii*, with tumor formation subsequent to flowering. No new terminal growth occurred adjacent to the tumorous tissue, but arose as shown from the basal parts of the plant and produced normal flowers. ($\times \frac{1}{10}$)

FIGURE 5. Dying plant of *N. debneyi-glauca* \times *N. langsdorffii* with abnormal tissue on the stems and roots. There is a mass of decomposing tumor tissue at the base of the stem. ($\times \frac{1}{10}$)



tumor development. This interpretation is supported by the fact that several hybrids that Kostoff (1943) described as forming stem tumors, in this study formed only root tumors (e.g. *N. glauca* × *N. plumbaginifolia*).

In this study tumors were found in nineteen interspecific combinations as shown in table 2, sixteen of which have never before been reported.

TABLE 2
INTERSPECIFIC GENOME COMBINATIONS OF NICOTIANA IN WHICH GENETICALLY-CONTROLLED TUMORS WERE FOUND IN THIS STUDY

Female parent	Somatic chromosome number	Male parent	Somatic chromosome number	Part of plant affected
* <i>N. debneyi</i>	48	<i>N. plumbaginifolia</i>	20	Roots only
* <i>N. debneyi-glauca</i>	72	<i>N. alata</i>	18	Roots only
* <i>N. debneyi-glauca</i>	72	<i>N. langsdorffii</i>	18	All parts
* <i>N. debneyi-glauca</i>	72	<i>N. plumbaginifolia</i>	20	Roots only
* <i>N. debneyi-glauca</i>	72	<i>N. sanderae</i>	18	Roots only (1 plant all parts)
* <i>N. debneyi-plumbaginifolia</i>	68	<i>N. glauca-langsdorffii</i>	42	All parts
* <i>N. debneyi-tabacum</i>	96	<i>N. alata</i>	18	Roots only
* <i>N. debneyi-tabacum</i>	96	<i>N. langsdorffii</i>	18	All parts
* <i>N. debneyi-tabacum</i>	96	<i>N. plumbaginifolia</i>	20	Roots only
* <i>N. debneyi-tabacum</i>	96	<i>N. sanderae</i>	18	Roots only
* <i>N. debneyi-tabacum</i> × <i>N. debneyi</i>	72	<i>N. debneyi-tabacum</i> × <i>N. debneyi</i>	72	All parts (1 plant in pop. of 12 plants)
<i>N. glauca</i>	24	<i>N. langsdorffii</i>	18	All parts
<i>N. glauca</i>	24	<i>N. plumbaginifolia</i>	20	Roots only
* <i>N. glutinosa-glauca</i> × <i>N. glauca</i>	36	<i>N. glutinosa-glauca</i> × <i>N. glauca</i>	36	All parts (1 plant in pop. of four)
<i>N. langsdorffii</i>	18	<i>N. glauca</i>	24	All parts
* <i>N. plumbaginifolia</i>	20	<i>N. glauca</i>	24	Roots only
* <i>N. suaveolens</i>	32	<i>N. sanderae</i>	18	Roots only (a few plants formed top tumors)
* <i>N. tabacum-glauca</i>	72	<i>N. alata</i>	18	Roots only
* <i>N. tabacum-glauca</i>	72	<i>N. sanderae</i>	18	Roots only

*Not previously reported.

POSSIBLE EXPLANATIONS OF THE CAUSATIVE AGENT

Three theories that have been advanced to explain the cause of genetically controlled tumors are: (1) immunological relationships, (2) heterogeneity of tissues and abnormal mitoses, and (3) cytoplasmic disturbances. The writer proposes a fourth theory based upon abnormal phytohormone relationships. It is necessary to rule out a virus, bacterium, or other pathogenic entity as a causative agent since all attempts over a period of years and by many different workers have failed to isolate an environmental causative agent from the genetic tumors on *Nicotiana* hybrids tested. In like manner, attempts to transmit an entity by grafting experiments from tumor-forming plants to normal plants have failed. Seedlings grown in the greenhouse on

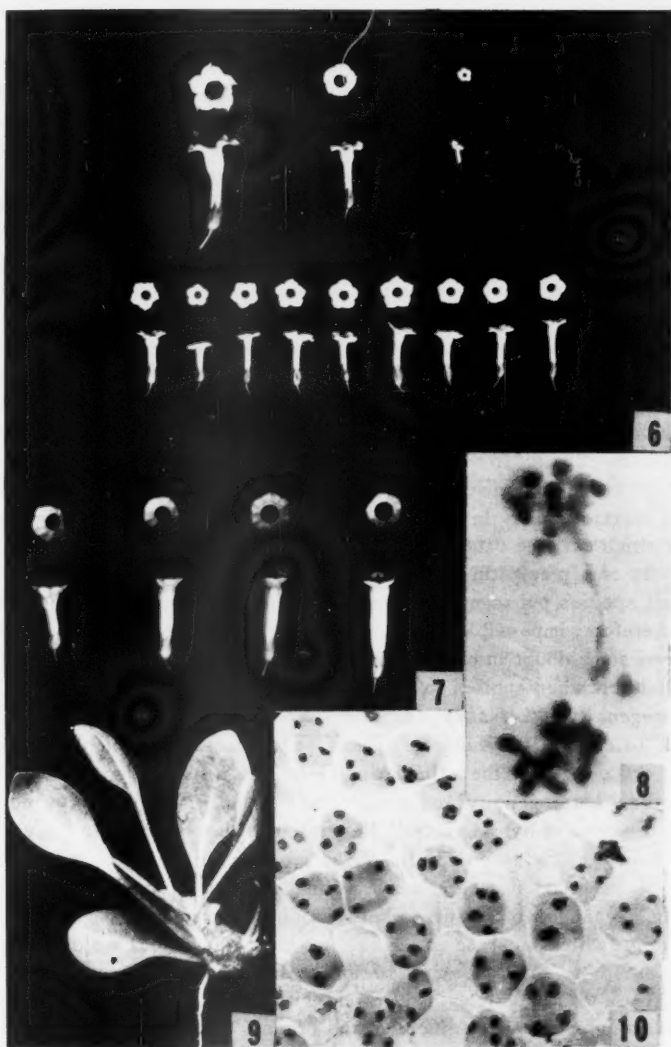


FIGURE 6. Corollas of plants combining genomes of *N. debneyi* and *N. tabacum*. Top row, left to right—*N. tabacum*, amphidiploid *N. debneyi-tabacum*, *N. debneyi*. Bottom row, plants with two genomes of *N. debneyi* plus random segregating chromosomes of *N. tabacum*, ($\times \frac{1}{4}$).

FIGURE 7. Corollas of plants combining genomes of *N. glauca* and *N. langsdorffii*. Right to left, LL (diploid *N. langsdorffii*), GLL, GGGL (amphidiploid *N. glauca-langsdorffii*) and GGGL, ($\times \frac{1}{6}$).

FIGURE 8. Photomicrograph of cell of the amphidiploid, *N. glauca-langsdorffii* at Anaphase I showing delayed separation of one bivalent. A few cells exhibit even more pseudo bridge-like structures, ($\times 3500$).

FIGURE 9. Seedling of the amphidiploid *N. glauca-plumbaginifolia* showing tumors, ($\times \frac{3}{8}$).

FIGURE 10. Photomicrograph of pollen mother cells of the amphidiploid *N. glauca-langsdorffii*, indicating the very normal meiotic divisions found in more than 99 per cent. of the cells, and the lack of any fragments in the cytoplasm, ($\times 260$).

steam sterilized soil readily formed tumors in the seedling stage indicating that the tumor causing factor must come with the seed. All available evidence to date indicates that the tumors described on *Nicotiana* hybrids are not the result of an environmental tumefacient agent and must be considered of genetic origin.

Immunological relationships: The theory of an immunological reaction as an explanation of tumor formation was first proposed in 1930 by Kostoff. In a later publication Kostoff (1943) did not mention the immunological reaction theory but proposed an entirely different explanation for tumors in the cross *N. glauca* \times *N. langsdorffii*, thus indicating that the immunological reaction theory had been discarded by its own author.

There are several phenomena that cannot be explained by this theory. *N. langsdorffii* can be used either as stock or scion in compatible grafts with *N. glauca*, and there is no evidence that tumor-causing substances diffuse across the graft to cause tumors in *N. glauca*. It is likewise difficult to explain by this hypothesis why tumors appear only when meristematic elongation is retarded, i.e., in the seedling stage and late blooming stage. In addition there are no experimental results which completely support the immunological reaction theory in *Nicotiana* hybrids other than that given by Kostoff (1933) which even he cautioned were only indicative. These data consisted of results of a precipitin reaction between the extracts of the paternal and maternal species put together *in vitro*. He did not give the method used and it is, therefore, impossible to repeat the experiment exactly. However, there has been some doubt in recent years concerning the validity of the precipitin test itself when applied to plant extracts exclusively.

Heterogeneity of tissues and abnormal mitoses: Kostoff (1943), in an attempt to explain the cause of tumors in the cross *N. glauca* \times *N. langsdorffii*, proposed that the stimulating factor for cell proliferation was most likely the "heterogeneity and mosaicism" of the plant tissues, "recalling the phenomenon of hybrid vigor." He explained that the mosaic tissue arose from "highly disbalanced" cells of the growing tip which in turn were the result of abnormal mitoses, and that in the apical promeristem there are somatic exchanges of segments between chromosomes of *N. glauca* and *N. langsdorffii* which increase in numbers as the plant becomes older. Quoting from Kostoff (p. 618), "Such genetically different cells, together with the normal ones that have remained, divide with various cell division rates and form tumorous instead of differentiated organs." Whitaker (1934) found no significant number of chromosomal irregularities in mitotic divisions of tumor cells that he examined, evidence which does not support this theory of Kostoff.

If there were abnormal mitoses in the cells of the apical promeristem, it would be logical to expect that meiosis would likewise be abnormal in the pollen mother cells differentiated from these same promeristem cells. Meiosis was studied in over 200 pollen mother cells from colchicine-induced amphidiploid plants which later formed tumors. In general all stages of reduction division were normal (fig. 10), and the chromosome numbers were

constant in all cells where chromosomes were counted. In less than one per cent. of the Anaphase I figures there was delayed separation of one of the bivalents, while in still fewer figures two or more bivalents seemed to be connected with chromatic strands, which on casual observation might be mistaken for bridges resulting from dicentric chromosomes. In figure 8 there were 20 chromosomes at each pole while the remaining pair of chromosomes seemed to be moving tardily to respective poles. In no cases were chromosome fragments observed remaining in the cytoplasm between the two poles at Anaphases I or II, as would be expected were the above condition a true chromosomal bridge. Further evidence of meiotic regularity was afforded by the uniformity of progeny of five lines of amphidiploids through two generations of selfing. Additional data in table 3 show that the five lines of amphidiploids and two backcrosses had a reasonably high amount of stainable pollen grains.

TABLE 3

STAINABLE POLLEN IN *Nicotiana glauca* × *N. langsdorffii* HYBRID COMBINATIONS

Line	Genomes involved	Designation	Size of pollen grains	% Stainable pollen
50191	*GLL	Amphidiploid	20-25 u	78.7
50192	GLL	Amphidiploid	17-25 u	94.8
50194	GLL	Backcross to <i>N. langsdorffii</i>	18-25 u	70.6
50195	GLL	Amphidiploid	20-26 u	90.5
50196	GLL	Backcross to <i>N. langsdorffii</i>	18-25 u	77.8
50197	GLL	Amphidiploid	18-24 u	88.5
50198	GLL	Amphidiploid	23-25 u	92.8
50211	LL	<i>N. langsdorffii</i>	18-25 u	88.3

*G = one *N. glauca* genome.*L = one *N. langsdorffii* genome

Kostoff reported that his amphidiploid, *Nicotiana glauca-langsdorffii* was not true-breeding, having as high as 55% deviates in the F₃, and only 51% stainable pollen (Kostoff, 1939). It is conceivable that he did not have a true amphidiploid though it is impossible to ascertain its origin. In three different publications he gives as many methods for producing the amphidiploid. In 1935 it was produced by crossing the triploid, (*N. glauca* × *N. langsdorffii*) × *N. langsdorffii* with *N. glauca* (Kostoff, 1935). In 1939 it was produced parthenogenetically by crossing the F₁ hybrid, *N. glauca* × *N. langsdorffii* with *N. langsdorffii*, and named *N. vavilovii* (Kostoff, 1939). In 1943 the origin is still obscure for he says "I doubled the chromosomes in the F₁ hybrids and produced self-fertile allopolyploids *N. glauca* × *N. langsdorffii* shortly called *N. vavilovii* (1935, 1938) which formed tumors like F₁ hybrids and represented very convenient objects for tumor investigations" (Kostoff, 1943, page 616). If this amphidiploid did not represent the two complete genomes from each parental species, it would explain not only why he found wide segregation in the progeny but also abnormal meiotic behavior. It does

not, however, fully explain the abnormal mitotic anaphases he reported in the root tips. This latter condition might well be only a step in a chain of reactions which ultimately cause tumor formations as a plant response to an unbalanced phytohormone mechanism.

The theory based on abnormal mitoses is also inadequate to explain why tumors appear in the early seedling stage before there has been opportunity for a build-up in the number of highly disbalanced cells.

Cytoplasmic disharmonies: Whitaker (1934) proposed that there was a cytoplasmic disturbance caused by the introduction of the chromosome complement of *N. langsdorffii* as a male into the cytoplasm of *N. glauca*. This was based in part upon his unconfirmed observation that the reciprocal cross did not produce tumors. The writer found tumors on hybrids of the reciprocal cross that were in all respects identical in appearance and development to those in the F_1 *N. glauca* (female) \times *N. langsdorffii* (male). Similar observations made in the cross *N. langsdorffii* (female) \times *N. glauca* (male) by Kostoff (1943) and Dr. H. H. Smith, Cornell University (unpublished), clearly establish the fact that the tumors found in both crosses are identical.

There is some additional evidence which makes even more unlikely Whitaker's cytoplasmic explanation. Tumors were formed in all parts of the plant produced by the cross of the amphidiploid *N. debneyi-glauca* \times *N. langsdorffii* (figs. 3 and 5). The amphidiploid was produced by doubling the chromosome set of the interspecific hybrid of *N. debneyi* \times *N. glauca*, so that the amphidiploid contained only the cytoplasm of *N. debneyi*. Yet when the chromosome complement of *N. langsdorffii* was introduced into this plant which had no *N. glauca* cytoplasm, tumors were formed. The cytoplasmic theory is, therefore, not supported by this later evidence and the writer knows of no subsequent experimental data which support it. Not only would it be difficult to explain tumor formation in all the hybrid combinations by this theory, but it would also be hard to reconcile the cytoplasmic theory with the established fact that tumors appear only when the plant is not growing rapidly.

Abnormal phytohormone relationships: The above three theories fail to explain completely the cause of spontaneous tumors found in the hybrids listed in tables 1 and 2. Consequently, the following five statements are marshalled in support of a more comprehensive theory of spontaneous tumor formation. All five statements are reasonably well substantiated by experimental data.

- (1) Phytohormone metabolism in plants is controlled by gene action.
- (2) *N. langsdorffii* possesses a genetically-controlled ability to make phytohormones less effective.
- (3) Phytohormones may produce tumors which are similar in many respects to those produced by chemicals, bacteria, viruses, and other environmental agents.
- (4) *N. langsdorffii* is more effective in tumor-forming hybrids than any other species of *Nicotiana* tested.

- (5) Plants produce tumors principally during a period of reduced terminal meristematic growth.

The above five statements will be discussed in the order given.

Statement one: Different species and varieties of plants have been shown to have heritable variations in their metabolism of auxin. Dwarf "nana" corn differs from normal-sized corn by a single gene, but is dwarfed in growth because there is less auxin available in its tissues for normal cell elonga-



FIGURE 11. Mass of root tumors on a plant of *N. debneyi-glauca* \times *sanderiae*. All indications are that these tumors, found in about 20 per cent. of the population, are early manifestations of the condition that later causes stem tumors, ($\times \frac{1}{4}$).

tion (Van Overbeek, 1935). Hybrids of *Epilobium* have an auxin production directly proportional to the size of the plant (Hinderer, 1936). The metabolic process affecting auxin production is the determining factor for the growth of the pea stem (*Pisum sativum*), the tall pea varieties having more available auxin than the dwarf forms (DeHaan and Gorter, 1936). These facts strongly indicate that inactivation or lack of function of phytohormones is controlled by gene action.

Statement two: Studies of the morphogenetic differences between *Nicotiana glauca* and *N. langsdorffii* show that the corollas follow the same general growth pattern but differ in cell elongation (Nagel, 1939). Corollas of *N. langsdorffii* are smaller than those of *N. glauca* chiefly because the cells are less elongated. There is evidence that growth regulators are inactivated in the corollas of *N. langsdorffii*. Equal concentrations of indoleacetic acid applied to the corollas of both species result in further cell elongation of

the *N. alata* corolla, but there is little or no response in that of *N. langsdorffii*. These experiments seem to indicate that the principal difference between the two species lies in the ability of each to utilize the growth regulator and that phytohormones are inactivated or do not operate effectively in *N. langsdorffii*. This characteristic is probably genetically controlled.

Statement three: Roots, stems, and buds all behave in a comparable way in that growth is inhibited by relatively high concentrations of auxins (Thimann, 1937). Furthermore, when indole-acetic acid is used at concentrations that inhibit growth, galls similar to those produced by crown gall bacteria are formed on a series of plants including *Nicotiana tabacum*. (Brown and Gardner, 1936; Friedman and Francis, 1942). It is a significant fact that wound phytohormones have been reported to cause spontaneous tumors in decapitated bean plants (Flint and Moreland, 1945). Thus it has been demonstrated frequently that relatively high concentrations of phytohormones produce tumors in plants, and under some conditions tumors have been reported to result from growth regulators generated within the plants.

Statement four: In an analysis of the sixteen tumor-forming hybrids listed in table 1, seven contain *N. langsdorffii* as one parent. Similarly, five of the nineteen hybrids found by the writer to produce tumors contain *N. langsdorffii* as one parent. A further analysis of the latter nineteen hybrids reveals that in those which produced tumors on all parts of the plant, all had *N. langsdorffii* as a parent, while the eleven hybrids which did not involve *N. langsdorffii* as a parent produced only root tumors, with the exception of three plants. It thus appears that *N. langsdorffii* is a more effective component in tumor-forming hybrid combinations than any other species of *Nicotiana* tested.

The tumor-forming ability of the *N. langsdorffii* genome remains effective even when overbalanced by additional genomes from *N. glauca*. Plants have been obtained with as many as three genomes of *N. glauca* to one of *N. langsdorffii*, (fig. 7). In all plants shown in table 4 characteristic tumors formed which were identical in all respects to those already described. Although the plants flowered successively later as the ratio of *N. glauca* genomes was increased, the tumors always appeared about four weeks after the first flower opened.

Additional evidence on the nature of the cause of tumors was found in two plants segregating for chromosomes of *N. tabacum* and *N. glutinosa* as shown in table 2. Both of these plants died soon after the appearance of the tumors.

The first was a plant out of a population of 12 segregants resulting from selfing the plant, *N. debneyi-tabacum* \times *N. debneyi*. All 12 plants more nearly approached the appearance and growth of *N. debneyi* than the backcross parent, but had varying degrees of *N. tabacum* characteristics (fig. 6). This would be expected as a result of the segregation of *N. tabacum* chromosomes. No tumors were noted either in the original amphidiploid, *N. debneyi-tabacum*, nor in the first backcross generation, *N. debneyi-tabacum* \times *N. debneyi*, nor in 11 of the above described segregants. Apparently the

TABLE 4
TUMOR-FORMING INTERSPECIFIC HYBRIDS BETWEEN *N. langsdorffii* AND *N. glauca* WITH VARYING GENOMES OF EACH SPECIES

Genome combi- nation	How produced	Number of genomes of <i>N. langsdorffii</i>	Number of genomes of <i>N. glauca</i>	Ratio of genomes of <i>N. langsdorffii</i> to <i>N. glauca</i>	Number of chromosomes of <i>N. langsdorffii</i>	Number of chromosomes of <i>N. glauca</i>	Ratio of <i>N. langsdorffii</i> chromosome to <i>N. glauca</i> chromosomes
GL*	<i>N. langsdorffii</i> × <i>N. glauca</i> and reciprocal	1	1	1:1	9	12	3:4
GLL	Amphidiploid <i>N. glauca-langsdorffii</i> × <i>N. langsdorffii</i>	2	1	2:1	18	12	3:2
GGL	Amphidiploid <i>N. glauca-langsdorffii</i> × <i>N. glauca</i>	1	2	1:2	9	24	3:8
GGLL	Colchicine doubling of the F ₁ interspecific hybrid	2	2	1:1	18	24	3:4
GGGL	Amphidiploid <i>N. glauca-langsdorffii</i> × 4N <i>N. glauca</i>	1	3	1:3	9	36	1:4

*G = *N. glauca* genome.

L = *N. langsdorffii* genome.

metabolism in these latter plants was such that the phytohormone relationship was not disturbed to the extent that tumors were initiated. However, the fact that tumors did occur in the one plant among the segregating population of twelve indicates that the ability for balanced cell division and tissue correlation was disrupted by the loss of certain *N. tabacum* chromosomes. As previously mentioned, gene action was found to regulate auxin concentration in dwarf nana corn (Van Overbeek, 1935) and undoubtedly phytohormone metabolism is likewise gene-controlled in *N. tabacum*. Thus, when the chromosome carrying this gene or genes is lost, control of the phytohormone regulatory mechanism would also be lost. The ultimate result would be a disturbance in phytohormone balance. Whether or not this is a correct explanation, the fact remains that tumor formation in the one plant described was associated with the loss of certain unidentified chromosomes of *N. tabacum*.

The second plant was one of four segregants in a population resulting from the selfing of *N. glutinosa-glauca* \times *N. glauca*, in which case it was the *N. glutinosa* chromosomes that were lost. This plant formed extremely large tumors, especially at the base of the stem.

Statement five: Another pertinent fact is that tumors appeared in the *Nicotiana* hybrids only during the stage of plant development when terminal meristematic growth in the stem was reduced. When the terminal buds were removed from actively growing plants, tumors readily formed at the tip of the stem and developed in other parts of the plant within 10 days, at least four weeks sooner than if the plant were allowed to grow normally. This was interpreted to indicate that there was a changed concentration of phytohormones when the apical cells were no longer actively elongating, and this altered concentration of phytohormones might be the condition initiating tumor formation.

All the above statements, considered as a whole, support the hypothesis that in certain genome or chromosome combinations of *Nicotiana* species, particularly those involving *N. langsdorffii*, the genetically-controlled phytohormone metabolism, which in the species themselves produces normal growth, is disturbed. This disturbance in the growth regulatory balance stimulates certain tissues of the hybrids to become meristematic resulting in the formation of undifferentiated masses of plant material, generally referred to as tumors.

SUMMARY

Studies were made of thirty-one interspecific tumor-forming hybrid combinations in *Nicotiana*, sixteen of which have not been previously reported. In these studies little evidence was found supporting three theories proposed prior to this study to explain the cause of genetic tumors. Such tumors are always spontaneously initiated from within the plant, are produced in entire populations of certain crosses, are transmitted to the progeny by factors carried with the seed, and occur only when the plant is not growing rapidly. Of all the species tested, *N. langsdorffii* was involved in more tumor-forming combinations than any other single species. In two

interspecific combinations tumor formation was associated with loss of chromosomes. Certain experimental data which cannot be explained by existing theories indicate that genetic tumors may have their origin in abnormal phytohormone relationships.

Species comprise harmonious systems in which developmental processes, including phytohormone utilization, are integrated to produce normal growth. However, in certain interspecific combinations of *Nicotiana*, particularly with *N. langsdorffii* as one parent, there is a disturbance of this growth regulatory mechanism. It is hypothesized that this results, at maturity and occasionally in the seedling stage, in the inability of the plant to normally metabolize phytohormones which in turn, results ultimately in a stimulation of certain tissues to become meristematic and to grow abnormally without definite cell differentiation.

ACKNOWLEDGEMENT

It is a pleasure to recognize the advice, cooperation, and encouragement of Dr. H. H. Smith, of Cornell University, in conducting this study and assisting with the manuscript. Credit is also given to Dr. Julian C. Miller, of Louisiana State University, for making part of this study possible.

LITERATURE CITED

- Brown, N. A., and F. Gardner, 1936, Galls produced by plant hormones, including a hormone extracted from *Bacterium tumefaciens*. *Phytopath.* 26: 708-713.
- DeHaan, I, and C. J. Gorter, 1936, On the differences in longitudinal growth of some varieties of *Pisum sativum*. *Rec. Trav. Bot. Neerl.* 33: 434-446.
- Flint, L. H., and C. F. Moreland, 1945, Note on gall formation in decapitated young bean plants. *Plant Physiol.* 20: 453-456.
- Friedman, B. A., and T. Francis, Jr., 1942, Gall formation by *Phytoplasma tumefaciens* extract and indole-3-acetic acid in cultures of tomato roots. *Phytopath.* 32: 762-772.
- Hinderer, G., 1936, Versuche zur Klärung der reziproken Verschiedenheiten von *Epilobium Bastarden*. *Jahrb. wiss. Bot.* 82: 669-686.
- Kostoff, D., 1930, Tumors and other malformations on certain *Nicotiana* hybrids. *Zent. Bakt. Parasit.* II 81: 244-260.
- 1933, Tumor problems in the light of researches on plant tumors and galls and its relation to the problem of mutation. (A critical review from biophysical, biochemical, and cytogenetical point of view.) *Protoplasma* 20: 440-456.
- 1935, Heritable tumors in plants experimentally produced. *Genetica* 17: 367-376.
- 1939, Studies of polyploid plants XXI. Cytogenetic behavior of the allopolyploid hybrids *Nicotiana glauca* Grah. \times *Nicotiana Langsdorffii* Weim. and their evolutionary significance. *Jour. Gen.* 37: 129-209.
- 1943, Cytogenetics of the genus *Nicotiana*. State's Printing House, Sofia, pp. 1-1073.
- Nagel, Lillian, 1939, Morphogenetic differences between *Nicotiana alata* and *N. langsdorffii* as indicated by their response to indoleacetic acid. *Ann. Mo. Bot. Gard.* 6: 349-374.
- Thimann, K. V., 1937, On the nature of inhibitions caused by auxin. *Am. Jour. Bot.* 24: 407-412.

- Van Overbeek, J., 1935, The growth hormone and the dwarf type of growth in corn. Nat. Acad. Sci. Proc. 21: 292-299.
- Whitaker, Thomas, 1934, The occurrence of tumors on certain *Nicotiana* hybrids. Jour. Arnold Arboretum 15: 144-155.

HYBRIDIZATION IN THE ANNUAL SUNFLOWERS: *HELIANTHUS ANNUUS* × *H. ARGOPHYLLUS*¹

CHARLES B. HEISER, Jr.

Indiana University

Helianthus annuus, the common sunflower, hybridizes freely with other annual sunflowers. Hybridization of *H. annuus* with *H. petiolaris*, *H. Bolanderi* and *H. debilis* var. *cucumerifolius* has been described previously, (Heiser, 1947, 1949, 1950), and in the present paper natural and artificial hybrids of *H. annuus* × *H. argophyllus* are analyzed.

*Helianthus argophyllus*² occurs today in both Texas and Florida. The species was originally described by Torrey and Gray in 1842 from material collected in Texas, and the earliest collection which I have seen from Florida is dated 1893 (Rolfs, University of Florida). It has been known in cultivation as the "silver leaf sunflower" since 1889 (Burpee Seed Catalogue). In all probability it was originally native to Texas but has escaped from cultivation to become naturalized in Florida. In Texas it is found only in or near the southern coast (fig. 1) in regions of sandy soil. This species is one of the slowest growing and latest blooming of the annual sunflowers, generally coming into flower in late August or early September.

The common sunflower, *H. annuus*, is today widespread throughout much of North America and is not uncommon in southern Texas (fig. 1). This species seldom or never occurs on sandy soil and generally comes into bloom in late June or July. The differences between these two species in soil preference and in time of blooming are strong barriers to their hybridization. However, the two species do come into contact in nature and there is an overlap in blooming seasons since *H. annuus* does flower into September.

The two species are closely related judging from their morphology. The most conspicuous difference is found in the pubescence—the leaves, stems, and bracts of *H. argophyllus* are covered with long silky hairs giving the plant a silvery gray color, whereas the pubescence in *H. annuus* consists of short, rough hairs and the plant is green in color. The leaf in *H. annuus* is always serrate, and in *H. argophyllus* it is nearly entire or shallowly

¹This project has been aided by grants from the Penrose Fund of the American Philosophical Society and the Graduate School of Indiana University. I should also like to extend my thanks to the curators of the following herbaria—Gray Herbarium of Harvard University, University of Florida, Missouri Botanical Garden, New York Botanical Garden, Southern Methodist University, University of Texas, Texas A. & M. College, and the United States National Herbarium—for making their specimens of the annual sunflowers available for study.

²Watson (1929) lists the original collection of *H. tephrodes* Gray in synonymy under *H. argophyllus*. I have seen the type specimen of *H. tephrodes* and it is clearly not *H. argophyllus*. The specimen, moreover, comes from California which is outside of the range of *H. argophyllus*. *Helianthus tephrodes* has been referred to *H. niveus* (Benth.) Brandegee by Blake (1918, p. 187).

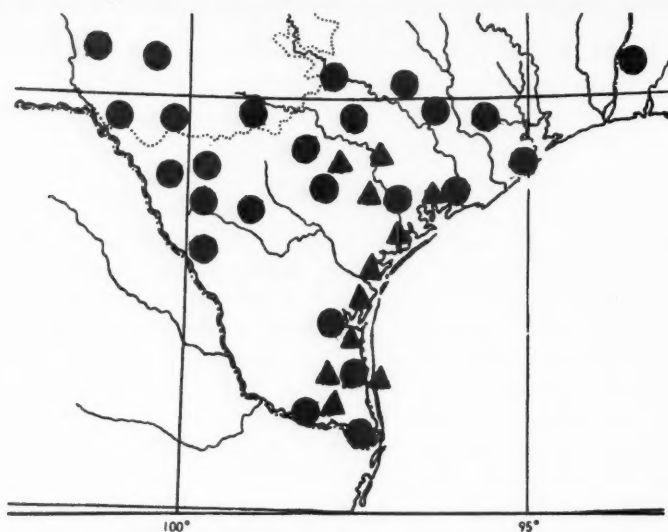


FIGURE 1. Map showing the distribution of *Helianthus annuus* (dots) and *H. argophyllus* (triangles) in southern Texas.

serrate. The achene in *H. annuus* is plump, in *H. argophyllus* it is rather flat.

NATURAL HYBRIDS

Putative natural hybrids between the two species have been collected in several localities in Texas by Dr. B. C. Tharp and by the writer (table 1). The specimens collected by Dr. Tharp are deposited in the herbarium of the University of Texas, and those of the writer are at Indiana University. The plants which are considered hybrids are intermediate between the parent species in pubescence, and match very closely the artificial hybrids which have been grown. In addition, the pollen fertilities of the putative natural hybrids show the same general range as those of the artificial hybrids (see below).

TABLE 1
PUTATIVE NATURAL HYBRIDS BETWEEN *H. annuus* AND *H. argophyllus* IN TEXAS

Collector and number	Locality	Pollen fertility
Tharp 1417	Kenedy Co.	33 per cent
Tharp 1639	Calhoun Co.	10 per cent
Tharp 48385	Kenedy Co.	25 per cent
Tharp 48385A	Kenedy Co.	17 per cent
Heiser 3072	Wilson Co.	25, 33, 40 and 42 per cent

The four hybrid plants collected by the writer along a roadside in Wilson County on June 17, 1949, were just coming into flower. Plants of *H. annuus* growing with the hybrids were in full bloom. In a field nearby where *H. argophyllus* grew in abundance a few of these plants were in bud although none was in flower on this date.

Neither at this locality nor at any other locality visited in eastern Texas were plants observed which appeared to be backcrosses. Either natural backcrosses are rare, or so similar to the recurrent parent in appearance that they are not readily detected in the field.

SPONTANEOUS AND ARTIFICIAL GARDEN HYBRIDS

History. Satsyperov (1916) reported on the cross of *H. annuus* \times *H. argophyllus*. Using a cultivated form of *H. annuus* as the female parent he obtained seed for an F_1 generation, but he did not discuss the relative fertility of the hybrids. Cockerell (1916) called attention to the slow growth of *H. argophyllus* and mentioned that this "peculiarity is dominant in a cross with *H. annuus* \times *lenticularis* (sic)." In 1918 he reproduced a photograph of a second generation cross between a red-rayed *H. annuus* and an *H. argophyllus*. In 1929 he again discussed this hybrid and stated that he found one hybrid with "about half of the pollen grains normal," and another with "hardly 10 per cent" normal pollen. Blakeslee (1945, p. 571) has also produced the hybrid between these two species.

F_1 hybrids. (4817). Two plants of *H. argophyllus* (seed from a herbarium specimen in the University of California, Cory 51242, San Patricio Co., Texas) were grown in an experimental field at Davis, Calif., along with plants of *H. annuus*. Open pollinated seed from *H. argophyllus* were planted the next year, and five of the eight plants secured were intermediate morphologically between *H. annuus* and *H. argophyllus*. The pollen fertilities of these five plants ranged from 10 to 33 per cent with a mean of 22 per cent. Upon open pollination the seed set on these plants ranged from a few seeds per head (about 5 per cent) to nearly 50 per cent. The remaining three plants grown which appeared to be pure *H. argophyllus* had pollen fertilities of 76 per cent or better and seed sets of over 90 per cent.

(3101). In 1948 plants of *H. argophyllus* and the red-rayed form of *H. annuus* were grown in a home garden. One plant which volunteered the next year was intermediate between the two presumed parents in pubescence but had the deep maroon rays approaching those of the *H. annuus* parent. The pollen fertility of this plant was 30 per cent and the seed set upon open pollination was about 10 per cent.

(4963). Reciprocal crosses made between *H. annuus* (seed from St. Louis, Mo.) and *H. argophyllus* (Bodger Seed Co.) produced 100 per cent seed set. The F_1 seedlings from the seed of the *H. annuus* parent died from some unknown cause soon after transplanting into the field, but five of the plants secured from the *H. argophyllus* parent reached maturity and were extremely vigorous. These F_1 plants bloomed in late July and early

August, intermediate between the blooming dates of the parental strains. The mean pollen fertility of these five plants was 33 per cent with a range of 30 to 36 per cent. Seed set upon open pollination varied from about 10 to 40 per cent.

Backcrosses. Backcrosses of 3101 were made to *H. argophyllus*. The pollen fertilities of the backcrosses were considerably higher than that of the F_1 , ranging from 49 per cent to 64 per cent with a mean of 58 per cent. In appearance these plants were almost indistinguishable from the recurrent parent in pubescence, but 4 of the 6 plants showed some red in the rays which was derived from genes of *H. annuus*.

Backcrosses of the artificial hybrid (4963) were made to both parents. The backcrosses to *H. argophyllus*, 29 in number, showed some variation in the degree of pubescence approaching the F_1 on one hand and the recurrent parent on the other (fig. 2). The majority of the plants, however, could not be distinguished from the recurrent parent unless the plants were compared side by side. The pollen fertilities on these plants averaged 47 per cent with a range of 25 to 76 per cent. Seed set was studied in two plants and varied from 10 per cent to approximately 50 per cent. These backcrosses all came into bloom earlier than *H. argophyllus* but were two weeks or more later in flowering than the backcrosses to *H. annuus*.

Six plants of the backcross to *H. annuus* were grown. The pubescence of these plants was always greater than that of the recurrent parent (fig. 2), but not outside of the range of pubescence found in *H. annuus* as a whole. For example, plants of *H. annuus* which are fully as pubescent as these backcrosses have been grown from seed from both Missouri and North Dakota, and it seems unlikely that the pubescence of the plants in these regions could have been derived from hybridization. The pollen fertilities of these backcross plants were slightly less than that of the parent species, ranging from 15 to 99 per cent with a mean of 63 per cent. Seed set on two plants was only slightly less than that normally found in the "pure" species. These plants all came into bloom two to three weeks later than their *H. annuus* parent.

CYTOLOGICAL OBSERVATIONS

Both *H. annuus* and *H. argophyllus* have the haploid chromosome number of 17 (Heiser, 1949). Meiosis was studied in five F_1 plants (table 2). In the majority of the cells there were 13 to 15 bivalents and one or two chains, rarely rings, of four chromosomes (fig. 3). In addition to the configurations given in table 2, univalents were observed in six cells, and in a few other cells chains of six or eight chromosomes were seen. No evidence of bridges and acentric fragments was observed at anaphase. From these results it would seem probable that the two species differ by two translocations. The number of pairs formed in this hybrid is much higher than in any of the annual hybrids of *Helianthus* previously studied (Heiser, 1947, 1949, 1950).

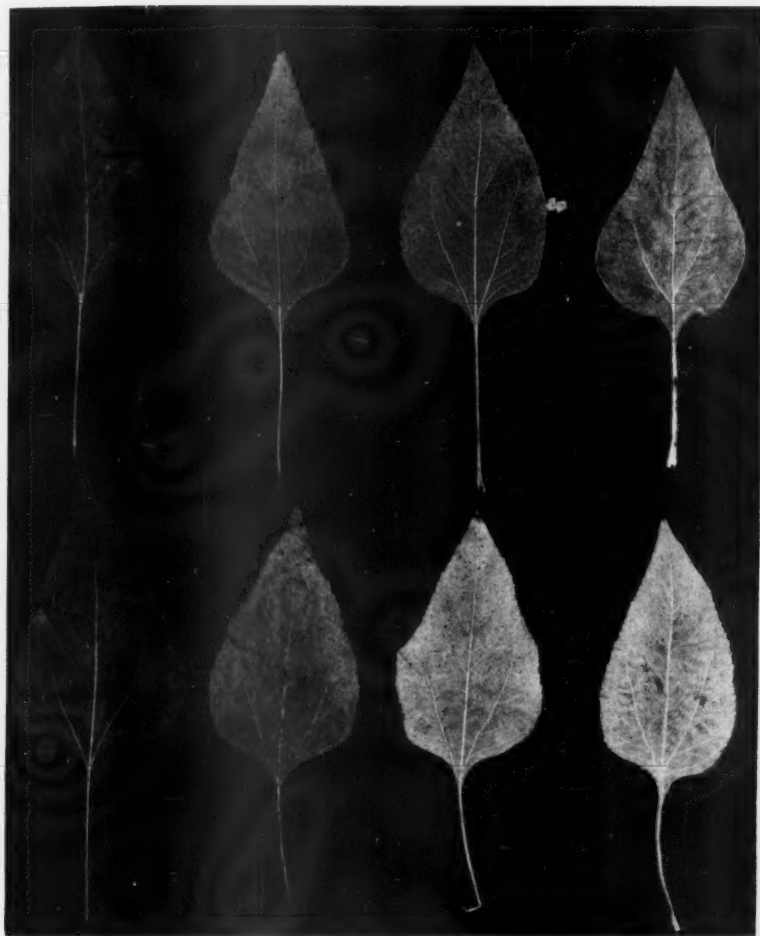


FIGURE 2. Leaves from greenhouse plants of *Helianthus annuus*, *H. argophyllus*, and backcross plants. First column, *H. annuus*; second column, *H. (annuus × argophyllus) × annuus*; third column, *H. (annuus × argophyllus) × argophyllus*; fourth column, *H. argophyllus*. (reduced)

DISCUSSION

Helianthus annuus and *H. argophyllus* are obviously species of close relationship as is seen by both the morphological and cytogenetic analyses. As yet little is known of the origin of these two species, although it seems likely that they came from a recent common progenitor and have differentiated in geographical isolation. In all probability their distribution was originally

TABLE 2

PAIRING IN F₁ HYBRIDS OF *H. annuus* × *argophyllus*

Configurations observed	Number of cells in hybrid					Total
	#4817-1	#4817-2	#4963-1	#4963-5	#3101	
17 II's	3	4	3	3	1	14
15 II's, 1 IV	15	6	3	8	16	48
13 II's, 2 IV's	2	9	1	2	9	23

allopatric. Through man's agency *H. annuus* was introduced into eastern Texas, and the two species have since hybridized.

Asa Gray (1884) stated that *H. argophyllus* "degenerates in cultivation apparently into *H. annuus*." Watson (1929), although realizing that *H. argophyllus* is a near relative of *H. annuus*, stated that there was no foundation for Gray's statement "that it hybridizes freely with *H. annuus*, or that it fades into the latter species along the northern limits of its range."

In all probability both Gray and Watson are correct to some extent, although Watson is attributing more to Gray than he actually wrote. In cultivation crossing may occur between these two species, and due to the fact that *H. annuus* is at an advantage in most garden soils it would seem likely that backcrosses to *H. annuus* would be the most likely to survive. Hence, *H. argophyllus* would "degenerate" into *H. annuus* through cultivation.

On the basis of limited field study and the study of herbarium material, no clearcut evidence of introgression in nature has been found, although it does seem possible that the occasional serrate leaf in *H. argophyllus* might have originally been derived from *H. annuus*. The hybridization experiments have shown that backcross plants approach very closely the recurrent parent in appearance. Moreover, in nature those backcrosses and other hybrid derivatives approaching the parental types would probably be most likely

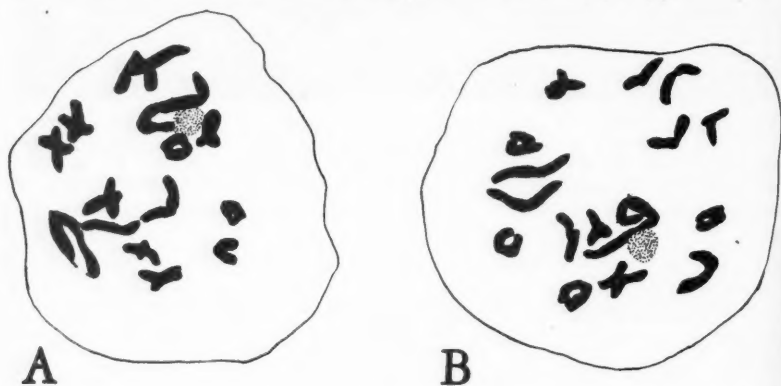


FIGURE 3. Camera lucida drawings of meiotic configurations in F₁ hybrid, *Helianthus annuus* × *argophyllus*. A, Cell showing 13 bivalents and 2 chains of 4 chromosomes each; B, Cell showing 15 bivalents and 1 chain of 4 chromosomes. X, 1280.

to survive. Hence, it would be exceedingly difficult to recognize the backcross plants in nature let alone to recognize introgressants. As Anderson (1949, p. 2) has written, "In all of the cases with which I am personally familiar, many, if not most, first and second backcrosses, if found in nature, would by taxonomists be accepted as varieties or slightly aberrant individuals of the species to which they were backcrosses." This statement certainly holds true for the backcrosses of *H. annuus* and *H. argophyllus*. It seems rather anomalous that these species show a higher degree of fertility than other annual hybrid combinations and at the same time the evidence for introgression is far less clearcut.

A third annual sunflower, *H. debilis* var. *cucumerifolius*, also occurs in eastern Texas, and like *H. argophyllus* is adapted to sandy soils. This species is known to hybridize with *H. annuus* (Heiser, 1950). No hybrids have been reported between *H. argophyllus* and *H. debilis* var. *cucumerifolius* although as yet critical field work has not been undertaken in southeastern Texas where such hybridization might be expected. The artificial cross between these two species has been made. This hybrid is very vigorous but rather highly sterile, producing from 1 to 21 per cent good pollen. In view of the fact that *H. argophyllus* and *H. debilis* var. *cucumerifolius* show the same general type of soil preference and occur in the same region, there must be other types of barriers preventing free hybridization between them. The difference in time of blooming between these species is rather pronounced, although there is some overlap, and probably is a rather potent barrier to extensive hybridization. The high degree of sterility of the F_1 hybrid, of course, would limit the amount of backcrossing. There may be other ecological barriers in addition, as yet unknown, which separate the two species.

If, as has been assumed, *H. annuus* is a recent immigrant in eastern Texas, its ability to spread and maintain itself in this region to which it was not adapted may be due to the acquisition of genes from *H. debilis* var. *cucumerifolius*, as previously suggested (Heiser, 1950), or from *H. argophyllus*. Watson is correct in stating that there is no evidence that the two species fade into one another.

No swamping is occurring as the result of the hybridization which is taking place because of the barriers imposed by the adaptation to different soil types, the partial sterility of the hybrids, and the partial difference in time of blooming. The ability of these entities to remain distinct in nature in spite of hybridization rightfully entitles them to the designation of species.

SUMMARY

Helianthus annuus and *H. argophyllus* are closely related species and natural hybrids between the two have been found in Texas. The artificial hybrid has been analyzed, and although showing some sterility, is more fertile than previously studied hybrids between other annual sunflowers. The artificial backcross plants approach very closely the recurrent parent in appearance, although they show some reduction in fertility. Analysis of

meiosis in the F_1 hybrid revealed 13 to 15 bivalents and one or two chains of four chromosomes in the majority of the cells. Whether introgression has occurred between these two species is not yet clear. Barriers imposed by the habitat, sterility and time of blooming have been effective in preventing the amalgamation of these two species under natural conditions.

LITERATURE CITED

- Anderson, E., 1948, Hybridization of the habitat. *Evolution* 2: 1-9.
Blake, S. F., 1918, A revision of the genus *Viguiera*. *Contrib. Gray Herb.* 54: 1-205.
Blakeslee, A. F., 1945, Removing some of the barriers to crossability in plants. *Proc. Amer. Phil. Soc.* 89: 561-574.
Cockerell, T. D. A., 1915, Specific and varietal characters in annual sunflowers. *Amer. Nat.* 49: 609-622.
1918, The story of the red sunflower. *Amer. Mus. J.* 18: 38-47.
1929, Hybrid sunflowers. *Amer. Nat.* 63: 470-475.
Gray, Asa, 1884, *Synoptical Flora of North America*. Vol. 1².
Heiser, C. B., 1947, Hybridization between the sunflower species *Helianthus annuus* and *H. petiolaris*. *Evolution* 1: 249-262.
1948, Taxonomic and cytological notes on the annual species of *Helianthus*. *Bull. Torrey Bot. Club* 75: 512-515.
1949, Study in the evolution of the sunflower species *Helianthus annuus* and *H. Bolanderi*. *Univ. Calif. Publ. Bot.* 23: 157-208.
1950, Hybridization in the annual sunflowers: *Helianthus annuus* \times *H. debilis* var. *cucumerifolius*. *Evolution*. In press.
Satsyperov, F. A., 1916, *Helianthus annuus* \times *H. argophyllus* A. Gray. *Bull. Appl. Bot.* 9: 207-244.
Watson, E. E., 1929, Contributions to a monograph of the genus *Helianthus*. *Pap. Mich. Acad.* 9: 305-475.

THE NEMATOCYSTS IN THE CTENOPHORE *EUCHLORA RUBRA*

Euchlora rubra (Kölliker) is known as the only ctenophore provided with nematocysts. As early as in 1856, Gegenbaur noticed the presence of nematocysts in this ctenophore. Chun (1880) also records and illustrates nematocysts in the same species in his magnificent monograph. Since then few workers, even authors of comprehensive works on Ctenophora, have mentioned this peculiarity in *Euchlora*. This is undoubtedly due to the extreme rarity of this ctenophore. In 1942 I had the chance of examining four specimens of this ctenophore collected at Seto in Wakayama Prefecture by Dr. T. Tokioka. On examining the sections of these specimens, I recognized numerous nematocysts in all of the specimens. The nematocysts were imbedded in the tissue of the tentacle along its lateral sides throughout the length. They were also found in the wall of the tentacular canal, on the side underlying the base of the tentacle. These nematocysts were of two kinds which might be called macro- and micronematocysts respectively. These occurred in mixture, macro- and micronematocysts roughly in proportion of 1:20-30. Thus, the occurrence in this ctenophore of the nematocysts is beyond question.

I reported this finding in two papers published in the same year. I had noticed, however, that the nematocysts were imbedded in the tissue of the tentacle, instead of being arranged on the surface of the latter. Especially, their occurrence in the wall of the tentacular canal appeared rather incomprehensible. More recently, at the suggestion of Dr. Libbie Hyman, I have become convinced that the nematocysts are of foreign origin, probably derived from some small medusae eaten by the ctenophore. This conjecture seems to clear up all the enigmas presented above.

It should be pointed out, however, that this ctenophore has no colloblast, and this feature is quite unique among tentaculate ctenophores. Possibly, this ctenophore uses its tentacles simply as balancing or floating apparatus, and captures its prey only with its large mouth. In any case, here is another case where nematocysts of foreign origin simulate their occurrence in an animal outside of the Cnidaria.

LITERATURE CITED

- Chun, C., 1880, Die Ctenophoren des Golfes von Neapel. Fauna u. Flora Golf. Neapel. Monogr. 1.
Gegenbaur, C., 1856, Studien über Organismen und Systematik der Ctenophoren. Arch. Naturg. 22: 163-205.

Komai, T., 1942, The nematocysts in the ctenophore *Euchlora rubra*. Proc. Imp. Acad. Tokyo 18: 255-256.

Komai, T. and T. Tokioka, 1942, Three remarkable ctenophores from Japanese seas. Annot. Zool. Japon. 21: 144-151.

TAKU KOMAI

NATIONAL INSTITUTE OF GENETICS
MISIMA, SIZUOKA-KEN, JAPAN

Lastly, the dactylozooids in the siphonophore *Porpita umbella* are arranged much like the tubuliform flowers of the Compositae on the head disc. Undoubtedly, the mode of arrangement of these zooids and flowers are subject to the same order.

LITERATURE CITED

- Komai, T., 1945, Order found in the arrangement of organs and zooids in some medusae. *Annot. Zool. Japon.* 23: 1-6.

TAKU KOMAI

NATIONAL INSTITUTE OF GENETICS
MISIMA, SISUOKA-KEN, JAPAN

PUBLICATIONS RECEIVED

THE AMERICAN NATURALIST is glad to acknowledge here the receipt of books on biological and natural history subjects which are likely to be of interest to our readers. No undertaking to publish reviews is implied in this acknowledgment. Books for notice may be sent to:

EDITORIAL OFFICE
The American Naturalist
635 W. 247 St.
New York 71, N. Y.

Arber, Agnes, 1950. *The Natural Philosophy of Plant Form*. 247 p. \$5. Cambridge University Press, American Branch, New York.

Bates, Marston, 1950. *The Nature of Natural History*. 309 p. \$3.50. Charles Scribner's Sons, New York.

Belkin, John N., 1950. Mosquitoes of the Genus *Tripteroides* in the Solomon Islands. 73 p. and 6 plates. *Proceedings of the U. S. National Museum*, Vol. 100. Washington, D. C.

Bent, Arthur Cleveland, 1950. *Life Histories of North American Wagtails, Shrikes, Vireos and Their Allies*. 411 p. and 48 plates. \$1.50. *Proceedings of the U. S. National Museum*, Bulletin 197. Washington, D. C.

Bessey, Earnst Athearn, 1950. *Morphology and Taxonomy of Fungi*. 791 p. \$7. The Blakiston Co., Philadelphia, Pa.

Braun, E. Lucy, 1950. *Deciduous Forests of Eastern North America*. 596 p. \$10. The Blakiston Co., Philadelphia, Pa.

Burton, Maurice, 1950. *Animals and Their Behavior*. 60c. Merlin Books, Edward Arnold and Co. London.

Clark, Frances N. and Anita E. Dougherty, 1950. *Average Lunar Month Catch by California Sardine Fishermen 1932-33 through 1948-49*. 28 p. Fish Bulletin No. 76. State of Calif. Dept. of Natural Resources, Division of Fish and Game. Bureau of Marine Fisheries.

Demerec, M., Editor, 1950. *The Biology of Drosophila*. 632 p. \$10. Contains articles by K. W. Cooper (Spermatogenesis); B. P. Sonnenblick (Early Embryology); D. F. Poulson (Differentiation of the Embryo); Dietrich Bodenstein (Postembryonic Development); G. F. Ferris (External Morphology of the Adult); Albert Miller (Internal Anatomy); Warren P. Spencer (Collection and Laboratory Culture). John Wiley and Sons, Inc. New York.

- Etkin, William, 1950. *College Biology*. 806 p. \$5. Thomas Y. Crowell Co., New York.
- Field, William D., 1950. *Moths of the Genus Cincia and Three New and Closely Related Genera*. 326 p. and 9 plates. *Proceedings of the U. S. National Museum*, Vol. 100, No. 3264. Washington, D. C.
- Friedmann, Herbert, 1950. *The Birds of North and Middle America*. Part XI. 793 p. *U. S. National Museum Bulletin* 50. Smithsonian Institution, Washington, D. C.
- Friedmann, Herbert, and Foster D. Smith, Jr., 1950. *A Contribution to the Ornithology of Northeastern Venezuela*. 127 p. and 2 plates. *Proceedings of the U. S. National Museum*, Vol. 100, No. 3268. Washington, D. C.
- Gibbs, R. Darnley, 1950. *Botany—An Evolutionary Approach*. 554 p. and 118 plates. \$6. The Blakiston Co., Philadelphia, Pa.
- Godsil, H. C. and Edwin K. Holmberg, 1950. *A Comparison of the Bluefin Tunas, Genus Thunnus from New England, Australia, and California*. 54 p. *Fish Bulletin* No. 77. State of California Dept. of Natural Resources, Division of Fish and Game, Bureau of Marine Fisheries.
- Jones, George N., 1950. *Flora of Illinois*. 368 p. University of Notre Dame Press, Notre Dame, Ind.
- Kerr, John Graham, 1950. *A Naturalist in the Gran Chaco*. 229 p. and 24 plates. \$4.50. Cambridge University Press, American Branch, New York.
- Lucas, Miriam Scott, 1950. *Elements of Human Physiology*, 2d Ed. 357 p. \$4.75. Lea and Febiger, Philadelphia, Pa.
- Maurain, Ch., 1950. *La Meteorologie et Ses Applications*. 258 p. 425 francs. Editor, Ernest Flammarion. Paris.
- Miller, Loye, 1950. *Lifelong Boyhood*. 226 p. \$2.75. University of California Press, Berkeley and Los Angeles.
- Moment, Gairdner B., 1950. *General Biology*. 680 p. \$5. Appleton-Century-Crofts, Inc., New York.
- Rand, Herbert W., 1950. *The Chordates*. 862 p. \$6. The Blakiston Co., Philadelphia, Pa.
- Sears, Paul B., 1950. *Charles Darwin*. 124 p. \$2. Charles Scribner's Sons, New York.

- Solis, M. Acosta, 1949. *Especies Forestales y Ornamentales Lenosas Aconsejadas para los Regiones y Climas del Ecuador*, Publication 7, 40 p. Departamento Forestal del Ecuador, Quito, Ecuador.
- Stebbins, G. Ledyard, Jr., 1950. *Variation and Evolution in Plants*. 643 p. \$8. Columbia University Press, New York.
- Titmus, F. H., 1949. *Encyclopedia of World Timbers*. 156 p. \$4.75. Philosophical Library, New York.
- Williamson, Henry, 1950. *The Phasian Bird*. 276 p. \$4. Little, Brown and Co., Boston.
- Wilson, Charles Branch, 1950. *Contribution to the Biology of the Philippine Archipelago and Adjacent Regions. Copepods Gathered by the U. S. Fisheries Steamer "Albatross" from 1887 to 1909, chiefly in the Pacific Ocean*. 441 p. U. S. National Museum Bulletin 100. Smithsonian Institution, Washington, D. C.
- Winge, Øjvind, 1950. *Inheritance in Dogs*. 153 p. \$3.50. Comstock Publishing Company, Ithaca, New York.

ERRATA

- Komai, Taku: In Sept.-Oct., 1950, issue first line, p. 391, should read: "schmidt, 1944, 'Science in the University,' pp. 183-210, gave several instances of crowd-"; on p. 392 the reference of Stern, C., should read, "1930. 'Multiple Allelie,' Handb. Vererb. 1."
- Gregg, John R.: In Nov.-Dec., 1950, issue, p. 419, line 4, insert "own" after "his"; p. 421, line 15, delete last word in line, "of"; p. 422, line 25, change "contrary" to "subcontrary"; p. 435, line 3, insert "and" after "obtained."



